River Assessment using Benthic Macroinvertebrates in the Hindu Kush-Himalaya Region

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Substrate and Current Preferences

and

Development of an Assessment Method

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"To be clear, an ecosystem is a community of animals and plants interacting with one another and with their physical environment. People are part of the ecosystem."

M. T. Barbour

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Glossary and Abbreviations

ASSESS-HKH: Development of an Assessment system to evaluate the ecological status of rivers in the Hindu Kush-Himalaya region. The project was funded by the European commission (contract number: INCO-CT-2005_003659). The objectives of the project were the development of tools for river monitoring with benthic macroinvertebrates.

CCA: Canonical Correspondence Analysis. Ordination method assuming a unimodal (bell-shaped) response of taxa over environmental parameters. CCA is a multiple regression method constraining biota samples on the observed environmental parameters.

Eigenvalue: Value that expresses the explanation shares of ordination axis or an environmental parameter in partial Canonical Correspondence Analysis (pCCA) to the total variability.

Ecosystem health: The term relates to ecosystem function. A healthy ecosystem contains a community which environment is not affected by anthropogenic activities.

EPT: Comprises benthic macroinvertebrates taxa of Ephemeroptera, Plecoptera and Trichoptera taxa. The abbreviation is often used in benthic macroinvertebrates related topics.

HKH region: Hindu Kush-Himalaya region. This thesis investigated the HKH countries Bangladesh, Bhutan, Nepal, India and Pakistan. The term HKH region is used synonymous for these five countries.

Integrative: To represent integration (integration: *lat.* integratio: to form a whole from differentiated).

Integrative assessment of rivers: Integrative assessment of rivers uses the biological community living in the river, e.g. the community of benthic macroinvertebrates to evaluate river health. The community integrates and indicates the impacts of many different stressor types on the ecosystem, such as organic pollution, eutrophication, hydromorphological degradation and land-use in the catchment area. The composition and the attributes of the community under reference condition serve as calibration for the assessment of other river sites. In this thesis the term “ecological river assessment” is synonymously used for integrative assessment of rivers.

Metric: Metrics are biological “attributes” which are firmly routed in sound ecological principles (Barbour *et al.* 1995). Metrics combine ecological functions or ecological demands that a group of taxa share, e.g. the same substrate preference, or comparable resistance to pollution. Metric values, e.g. number of “stone dwelling animals (“Lithal”)” or “number of Oligochaeta” can be used to describe and evaluate ecological processes. In terms of river assessment a metric is characteristically responsive to river degradation, and its alteration due to increased human influence is predictable and measureable.

Monte Carlo Permutations test: The null hypothesis assumes that the response of taxa is independent of the environmental parameters. Monte Carlo Permutations tests this hypothesis by randomly assigning (shuffling) the species compositions to the observed environmental parameters. The results of the test are compared with the “real” situation, and test statistics are calculated (Jongman *et al.* 2004).

Multimetric index: A multimetric index integrates the information of most suited metrics (core metrics) into a single value (index). By using many metrics (multimetric) the cumulative impacts of anthropogenic activities on the biological community are considered (e.g. organic pollution, eutrophication, hydromorphological degradation and land-use in the catchment area). The index of a certain sampling site is compared with the index under reference conditions. The deviation of the observed index from the reference index can be calibrated to a river quality class.

PCA: Principal Component Analysis. An ordination method where straight lines are supposed to best describe the variability along the ordination axes. PCA reduces redundant information in the data set as it calculates new axis (principal components), which summarize and show as much information as possible. Simultaneously, it detects the most important gradients of environmental parameters, reflected by the parameters arrow lengths in the ordination plot.

pCCA: partial CCA. The effects of several explanatory parameters (i.e. environmental parameters) are separated. The pCCA calculates the univariate regressions on each of the explanatory parameter.

Reference condition: A river stretch which is not affected by anthropogenic activities.

River quality assessment: River quality assessment evaluates the impacts of anthropogenic activities on the river ecosystem. The strength of the impacts depends on the exposure time and the intensity of a certain stressor. Quality assessment of a particular river stretch is conducted with respect to reference conditions. The degree of deviation from reference condition may be expressed as river quality class.

Stressor: Stressors are environmental parameters describing anthropogenic activities, and their increase or decrease lead to river deterioration. Different stressors may be summarized to a certain stressor type (Table 15). The thesis on hand investigates the four stressor types eutrophication, organic pollution, hydromorphological degradation and land-use in the catchment.

T-value biplot: A t-value biplot is a figure containing arrows for the species and symbols for the environmental parameters. It is used to reveal statistically significant pair-wise relationships between species and environmental parameters (i.e. which taxon depend on which environmental parameter). Using the biplot projections, one approximates the t-values of the regression coefficients (derived from the CCA) with the taxon acting as a response variable and all the environmental parameters as predictors. A t-value larger than two is assumed to be a critical value indicating significant relations between taxa and the environmental variable. This threshold value is displayed by the t-value biplot. Taxa that are positively correlated with environmental parameters are enclosed by the circle (Ter Braak & Smilauer 2002).

State-of-the-art

About 500 million people in South Asia depend directly on the abundant water from the mountainous rivers of the Hindu Kush-Himalaya. The rivers provide water for irrigation, drinking, household, municipal water use, industrial activities, and more than in other regions of the world dominate the religious and cultural life of the society. In spite of the integral role of water for the economic well-being of the area, rivers are under tremendous pressures; additionally monitoring of rivers and sustainable water uses are underdeveloped. In the Hindu Kush-Himalaya countries, approximately 20% of all deaths among children under five years of age is caused by water borne diseases (data for the year 2000, WHO 2008).

According to the current State of the Environment reports (SOE) from the investigated Hindu Kush-Himalaya countries Bangladesh, Bhutan, India, Nepal and Pakistan river deterioration derives from four major factors:

1. Waste: Sources are hazardous waste, industrial effluents, domestic effluents, agriculture effluents, solid waste, seaports-oil and lube spillage, and salinity intrusion.
2. Land-use: Main problems are rapid urbanisation, increase in slums, increase in industrial activities, and intensive use of agriculture land.
3. Damming/Impoundment: Problems related to irrigation and hydropower production.
4. Climate change: Global warming may lead to glacial lake outburst floods.

(SOE Nepal 2001, SOE Bangladesh 2002, SOE India 2002, PCRWR Pakistan 2002, SOE Bhutan 2004, and ICIMOD/ASSESS-HKH 2005) (Figure 1)

Of the aforementioned factors the most important stressors in topical areas are effluent of domestic discharge, untreated industrial and municipal discharges, and runoff pollution from agriculture (Central Pollution Control Board 2003a, 2003b, SAWAN 2005, Sharma *et al.* 2005, Hoffmann & Shrestha 2008). Consequently, current lack of adequate supply of clean and potable water is a severe problem in the

Hindu Kush-Himalaya region leading to impacts on human health and economic development (Hutton 2001, World Bank 2006).

However, all of the governments of Bangladesh, Bhutan, India, Nepal and Pakistan implemented or are currently planning to adopt water quality standards, national water development and management plans. A list of most important measures follows: *Bangladesh:* National Fisheries Policies 1998, Environment Conservation Act 1998, The National Policy for Safe Water Supply and Sanitation 1998, Environment Court ACT 2000. Source: SOE Bangladesh 2002. *Bhutan*: Environmental Assessment Act 2000, Environmental Clearance and Environmental Assessment Strategic 2002, Environment Discharge Standard 2004, Source: RGOB 2000, 2002, 2004a, 2004b. *India*: National Conservation Plan – the Ganga and the Yamuna Rivers 1995, Municipal Waste Rules 1999, National Water Policy 2002. Source: MOWR 2000, Central Pollution Control Board 2003a, 2003b. *Nepal:* Water Supply Corporation Act 1989, Drinking Water Regulation 1998, Nepal Water Resources Strategy 2002, Irrigation regulation 2003, National Water Plan 2005. Source: DWSS 2002, GON/UNDP 2002, MWR/WEC 2005. *Pakistan*: Environmental Protection Act 1997, National Environmental Quality Standards and Rules 2000, Source: PCRWR 2002, Kahlown & Azam 2004.

The requirements for water quality monitoring differ considerably between the countries. In *Bangladesh* the national standard for inland surface water is defined through physical, chemical and bacteriological parameters, pH, biological oxygen demand (BOD), dissolved oxygen (DO) and total coliform counts. In addition standards for sewage discharges, standards for waste from industrial projects and standards for drinking water quality exist, all based on abiotic parameters (Pradhan *et al.* 2005). *Bhutan* has no national standard for inland water, but does have drinking water standards and discharge standards for industry. The latter standards derive from physical, chemical and bacteriological parameters (Pradhan *et al.* 2005). In *India* the national standard of inland waters is assessed through pH, BOD, DO and total coliform counts. Furthermore there exist standards for sewage discharge, waste from industrial projects and drinking water quality which are defined through abiotic parameters (Pradhan *et al.* 2005). In addition the Central Pollution Control Board under the Ministry of Environment and Forest of India regularly conducts biological monitoring with focus on saprobity indicating organism. The assessment is based on the Biological Monitoring Party (BMWP) and the Average Score per Taxon (ASPT) (Armitage *et al.*

1983), and adapted to benthic macroinvertebrates of Indian rivers. *Nepal* has no national standard for inland surface water nor has it officially adopted any drinking water quality standard. However the National Task Force on Drinking Water Quality Standards (2005) proposes a number of physical, chemical and microbiological parameters for drinking water quality standards (state 2005) (Pradhan *et al.* 2005). Unlike this proposal, Nepalese scientists explored riverine ecology and developed a biological monitoring tool for Nepalese rivers and the Ganga river systems, also based on the BMWP and the ASPT (Sharma & Moog 2005, Nesemann *et al.* 2007). *Pakistan* has not adopted any national water quality standards. Currently, the Pakistan Council of Research in Water Resources has drafted physical, chemical and bacteriological standards for drinking water quality (state 2005). Since 2001 a national water quality monitoring programme investigates a net of rivers and observes physical, chemical and bacteriological parameters (Pradhan *et al.* 2005). Recently, Qadir & Malik (2009) developed a multimetric index based on fish to assess ecological river quality in rivers of Pakistan.

Thus, in the represented countries river assessment procedures, if implemented at all, mainly comprise measurements of physical and chemical parameters, pollutants, toxic substances, partly bacteriological parameters, and occasional biological monitoring with main emphasis on indicators of organic pollution. But, taking merely measurements of abiotic parameters to assess river quality has several disadvantages, and do not wholly reflect river health:

- Chemical and physical parameters are not the only parameters on which the health of the ecosystem depends.
- In terms of river quality assessment the interpretation of physical and chemical analysis is difficult.
- The measured value only reflects the situation of the river at a single location and at a particular time.
- Pollutants may interact and hence their impact may be strengthened.
- It is impossible to develop and apply analytical methods for each pollutant.
- Many pollutants which are not or not regularly monitored may still be of ecological significance.

- The carrying out of physical and chemical measurements is expensive in terms of the equipment needed and number of analysis needed to achieve reliable results.

(Resh 1995, Karr & Chu 1999, Dudgeon 2003)

In contrast to the previously listed monitoring methods benthic macroinvertebrates as bioindicators provide a more comprehensive estimate and are a valuable addition of conventional monitoring programmes. Benthic macroinvertebrates have long been used for quality assessment, thus spawning a variety of biological monitoring tools (Hellawell 1986, Cairns & Pratt 1993, Rosenberg & Resh 1993, Davis & Simon 1995, Karr & Chu 1999, Birk & Hering 2002, Hodkinson & Jackson 2005). Biological monitoring tools have various advantages:

- The community parameters of benthic macroinvertebrates (metrics) have a strong linkage to quality and intensity of various stressors.
- The benthic macroinvertebrate community provide reliable and relevant signals about the biological effects of human activities.
- The benthic macroinvertebrate community integrates the effects of stressors over time.
- Costs to implement monitoring programmes are low compared to physical and chemical monitoring programmes.

(Resh 1995, Dudgeon 2003, Barbour *et al.* 2004)

The basis for the development of biological assessment tools is the knowledge about ecological processes in rivers, e.g. about ecological demands of benthic macroinvertebrate taxa; especially if focusing on an integrative assessment of river ecosystems. Currently this knowledge is incomplete in the Hindu Kush-Himalaya region. Suitable information focus only on either saprobic classifications, and in addition is limited to India and Nepal (Central Pollution Control Board 1999, Sharma & Moog 2005, Nesemann *et al.* 2007), or feeding habits of several benthic macroinvertebrates (summaries in Dudgeon 1999, Yule & Sen 2004).

Mountain rivers

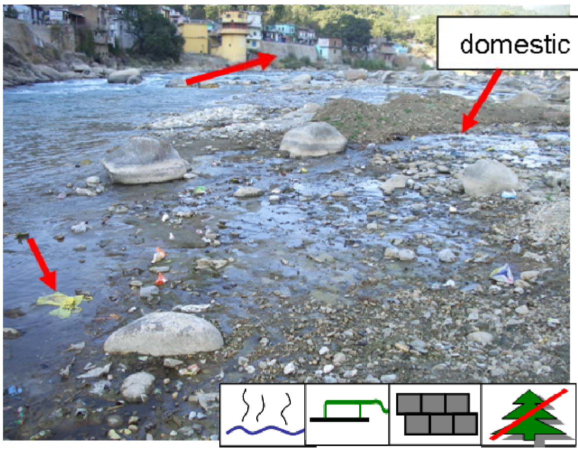


Figure 1a: Village, waste, bank fixation

Figure 1b: Domestic effluents

Figure 1c: Washing, bathing, toilet

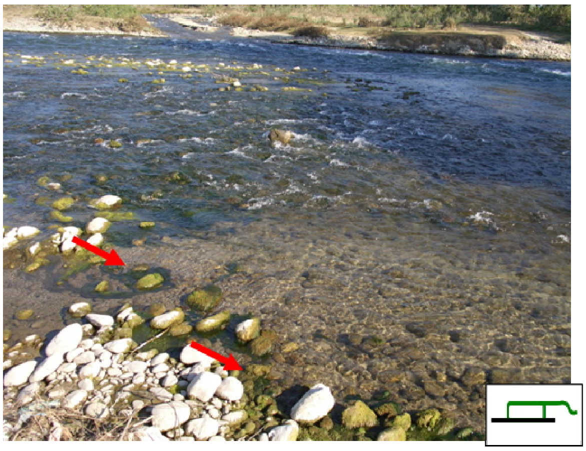


Figure 1d: Increased algae growth

Figure 1e: Weir, pollution

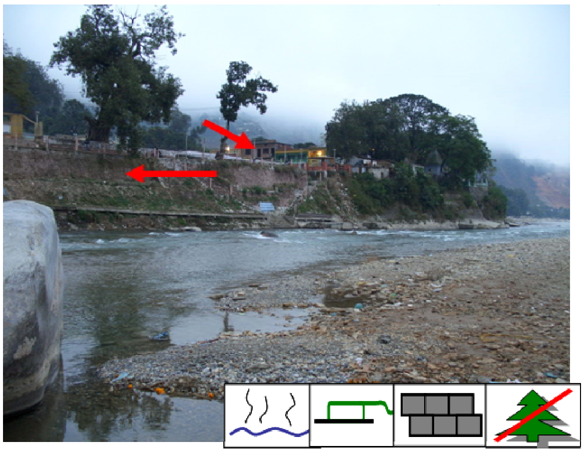


Figure 1f: Village, bank fixation, lack of wooded riparian vegetation

Mountain rivers

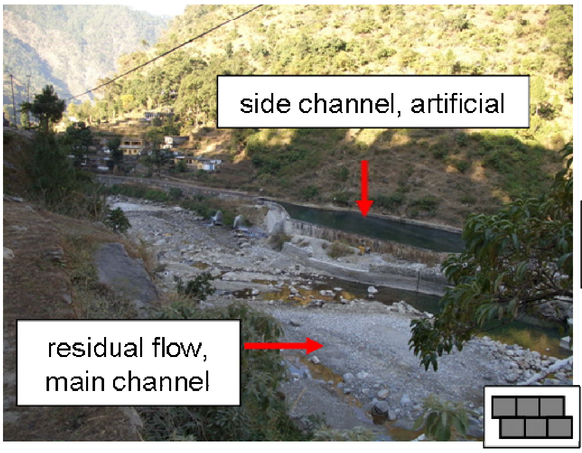


Figure 1g: Dam, water abstraction for power station

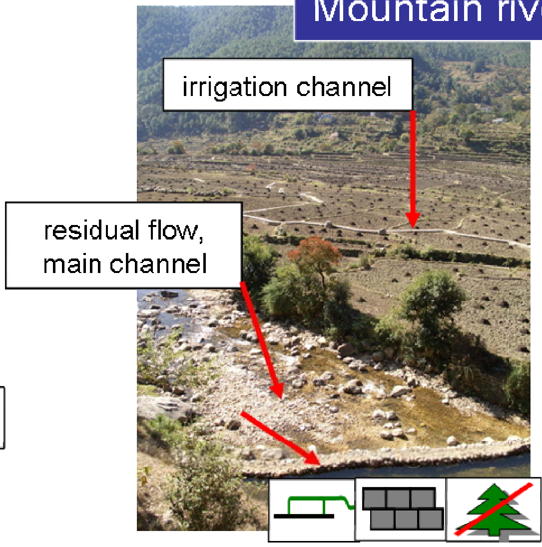


Figure 1h: Weir for irrigation, agriculture

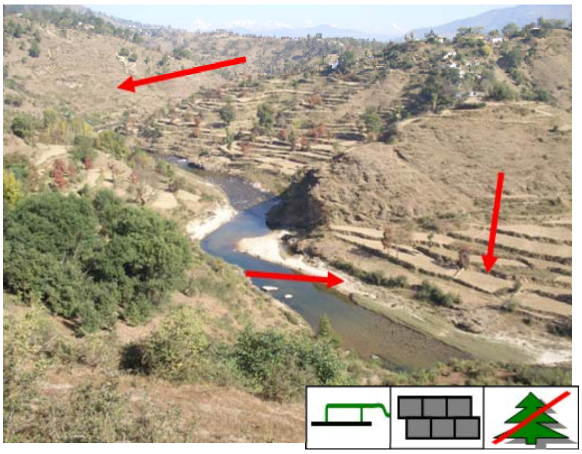


Figure 1i: Agriculture (terraces), lack of wooded riparian vegetation, lack of forest

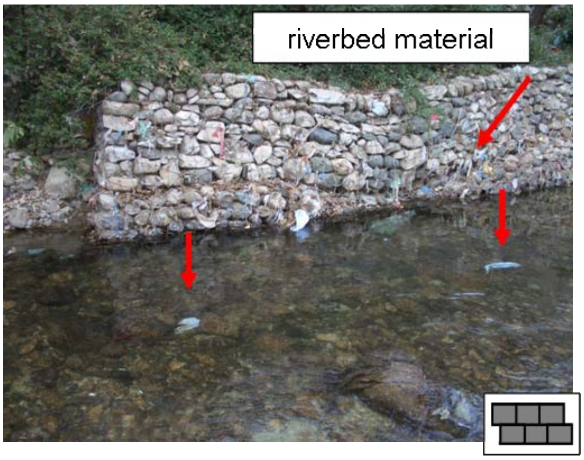


Figure 1j: Bank fixation, waste

Lowland rivers

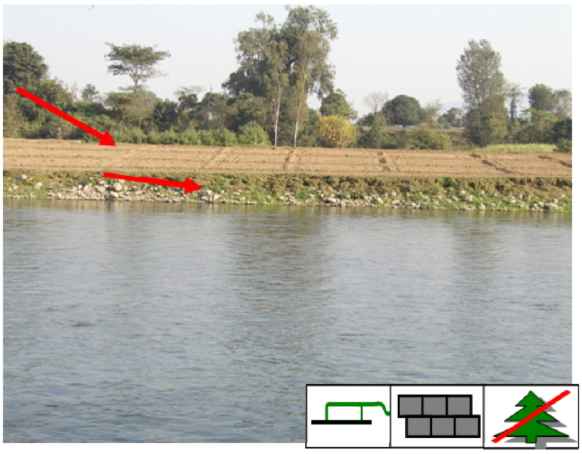


Figure 1k: Agriculture, lack of wooded riparian vegetation, bank fixation

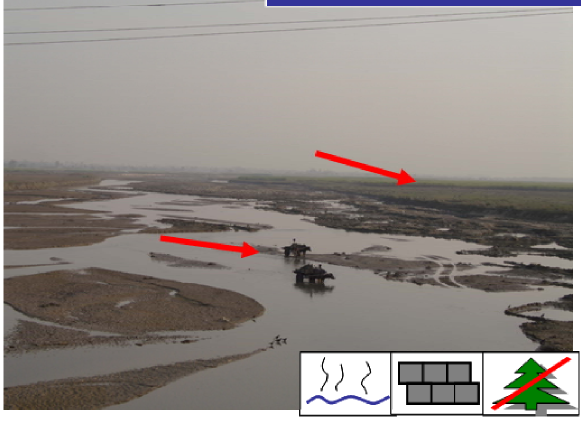


Figure 1l: Cattle watering place, river crossing, lack of floodplain vegetation

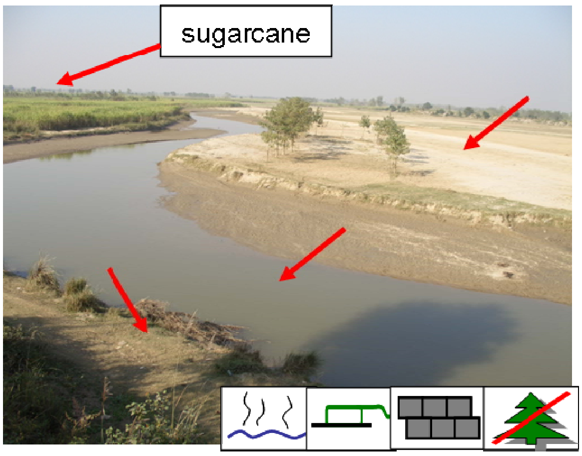


Figure 1m: Agriculture, lack of wooded riparian vegetation, lack of floodplain vegetation, pollution

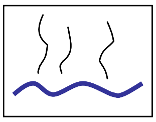
Figure 1n: Sugar cane factory

Figure 1o: Straightening, bank fixation, pollution

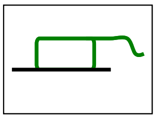
Figure 1p: Removal of mineral bed material

Figure 1a-p: Stressors of river ecosystems in the Hindu Kush-Himalaya region (examples from India). Date: Post-monsoon 2005, pre-monsoon 2006. Photos: Thomas Korte.

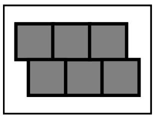
<u>Stressor types</u>



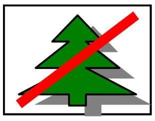
Organic pollution



Eutrophication



Hydromorphological degradation



Land-use of the catchment

Objectives and Scope of the Thesis

This thesis deals with the benthic macroinvertebrates of the rivers in the Hindu Kush-Himalaya located in Bangladesh, Bhutan, India, Nepal and Pakistan. It has two objectives. The thesis aims at expanding the knowledge on ecological preferences of benthic macroinvertebrates in the rivers of the Hindu Kush-Himalaya region as background information for their usage as bioindicators. Second, it aims to develop a biological monitoring tool for integrative river ecosystem assessment based on benthic macroinvertebrates.

Chapter 1 investigates the autecology of benthic macroinvertebrates in the rivers of the Hindu Kush-Himalaya region, in particular their substrate and current preferences. For selected taxa substrate and current preferences are further described through the development of a 20 point system. Metrics are developed which are suitable to detect hydromorphological impacts on river ecosystems.

Chapter 2 describes the development of a multimetric index assessment system to evaluate the ecological status of rivers in the Hindu Kush-Himalaya region using benthic macroinvertebrates. The procedure mainly follows the principals explained by Hering *et al.* (2006a).

1 Substrate and Current Preferences of Benthic Macroinvertebrates as Impact Indicators of Hydromorphological Degradation

1.1 Introduction

Ecological data on aquatic benthic macroinvertebrates are used worldwide to evaluate river ecosystems (Barbour *et al.* 1999, Usseglio-Polatera *et al.* 2000, Hering *et al.* 2004a, Baptista *et al.* 2007, Tomanova *et al.* 2008). These are usually based on literature evaluation of which required information about taxa is filtered and summarized (Merrit & Cummins 1996, Schmedtje & Colling 1996, Statzner *et al.* 1997, Barbour *et al.* 1999, Moog 2002, Eurolimpacs Consortium 2008). Taxa sharing information, e.g. on pollution tolerance, current or substrate preferences and life-cycle, may be combined into indices (Hilsenhoff 1987), metrics (Barbour *et al.* 1996) and traits (Townsend & Hildrew 1994). Indices, metrics and traits are able to describe and monitor ecological processes, and thus are used for ecological river assessment (Barbour *et al.* 1996, Dolèdec *et al.* 1999, Sandin & Hering 2004).

Another approach to obtain information on benthic invertebrates for river assessment is to conduct targeted investigations with suitable sampling designs and corresponding data analyses. However, this was rarely put into practice (Schmedtje 1995, Tomanova *et. al.* 2006); probably due to the fact that in North-America and Europe, where most assessment methods originate ecological information of benthic macroinvertebrates is sufficiently available. In regions were the knowledge about river life is scarce the development of ecological river assessment methods must follow the latter approach.

Rivers in the Hindu Kush-Himalaya region are currently under a variety of human stressors, such as pollution and hydromorphological degradation with the removal of mineral and woody river bed material or logging of wooded riparian vegetation (Messerli & Ives 1997, Sharma *et al.* 2005, Singh & Singh 2007). River assessment with benthic macroinvertebrates would provide an effective tool to detect the impacts of the latter river degradations (Karr & Chu 1999), though at the moment the underlying datasets are not available. Ecological information of benthic invertebrates in the Hindu Kush-Himalaya region is incomplete (Dudgeon 1999, 2003, Yule & Sen 2004), and if available relates only to pollution tolerance, rather than community health (Central Pollution Control Board 1999, Sharma & Moog 2005, Nesemann *et al*. 2007).

This part of the thesis investigates substrate and current preferences of aquatic macroinvertebrates for river assessment in the lower mountains and the lowlands of the Hindu Kush-Himalayan region, and covered the countries Pakistan, India, Nepal, Bhutan and Bangladesh. The results of the investigation are designed for use in practical river assessment. The investigation comprises the following working steps. A standardized substrate specific sampling was carried out to obtain biological data and explanatory environmental parameters. In a first step the observed environmental parameters were tested if they could explain the variability of the data. Then, statistical methods were applied to assign significant substrate and current preferences to certain taxa. Substrate and current preferences were quantified to provide the necessary background information for the investigated taxas' ecological potential to evade habitat alteration. In addition, substrate and current preferences of taxa were combined to different metric types. Metrics were tested as indicators of the impact of hydromorphological degradation. In order to provide a broadly reproducible study format, a set of threshold values for metrics were defined to detect stressed and unstressed sites.

1.2 Material and Methods

1.2.1 Study area, site selection and sampling

Geography

The Hindu Kush-Himalaya region covers 600,000 km² and includes parts of eight countries from Pakistan in the west to Myanmar in the east. Major river systems are the Indus, the Ganges and the Brahmaputra. The total mountain area drained by the Ganga and its tributaries is about 150,000 km². The Indus and the Brahmaputra each by itself drains more than 250,000 km². The landscape is characterized by a steep altitudinal gradient in south-north direction, including the Mount Everest in Nepal with elevation of 8,848 m. Three parallel zones of different altitudinal ranges subdivide the Hindu Kush-Himalaya region, each with a west-east extension of 3,500 km: the High Mountains (> 5,000 m above sea level) bordering the high plateau of Tibet, the Middle Mountains (2,000-5,000 m), and the Siwaliks (500-2,000 m). In the south, the area descends to the Indo-Gangetic Plains, which cover parts of India, Nepal and Bangladesh.

Climate and river hydrology

The climate of the Hindu Kush-Himalaya is ruled by the monsoon bringing precipitation with about 80% of annual rainfall from June to September. The monsoon is of orographic nature. The rainy season arises with south-westerly winds over India and rainfall increase with altitude to maxima in the Middle Mountains of the Hindu-Kush Himalaya region. Further north to the High Mountains rainfall decreases. Minimum rainfall occurs during the winter (post-monsoon) between October and February. March to June is considered as a transnational period when light to moderate rainfall can arise from local convective storms (pre-monsoon). The rivers discharge respond to the heavy precipitation in the rainy season. In the initial stage of the monsoon discharge remains low as catchments are unsaturated, but once soil storage are filled river discharge increase tremendously (e.g. Likhu Khola (Nepal), 2050 m: pre-monsoon discharge < 10-30 Liter/sec, monsoon discharge > 2 m^3/sec) (Brewin *et al.* 2000).

Site selection

Mountainous rivers in the lower parts of the Middle Mountains located in Bhutan, India and Pakistan, in the Siwaliks located in Bhutan, India, Nepal, and Pakistan, and lowland rivers in the northern parts of the Indo-Gangetic Plains (Bangladesh, India and Nepal) were investigated (Figure 2).

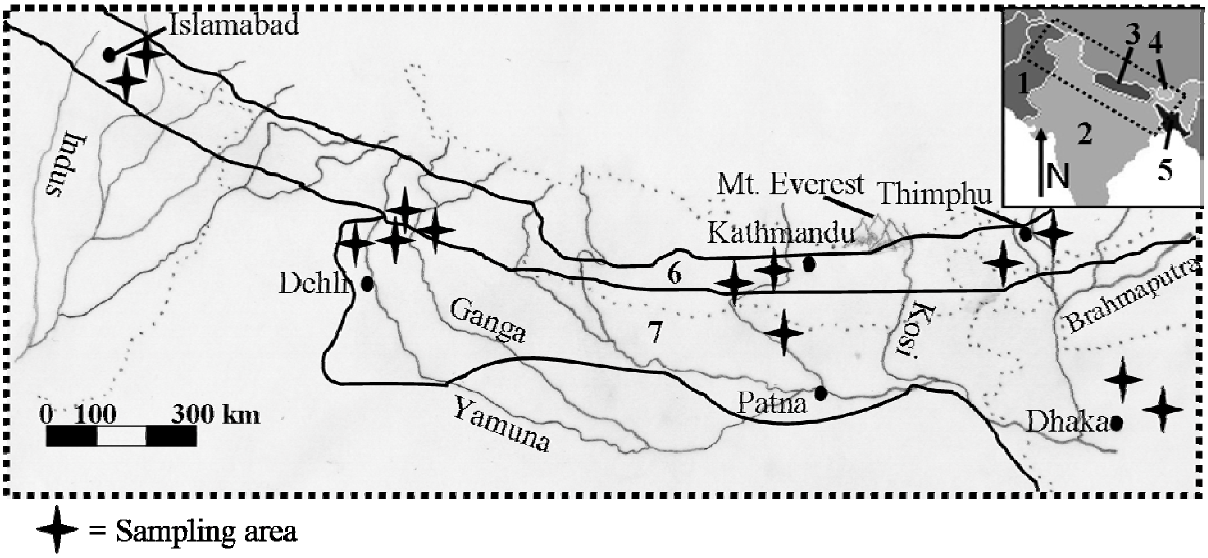


Figure 2: Study area: 1 = Pakistan; 2 = India; 3 = Nepal; 4 = Bhutan; 5 = Bangladesh; 6 = Middle Mountains, Siwaliks; 7 = Indo-Gangetic Plains.

Catchment size and altitude were comparable within the lower mountainous river group and within the lowland river group. All streams investigated are characterized by permanent flow, fed by sources and of small to medium size (i.e. wadeable in the dry season). Riverbeds of the lower mountainous region are dominated by large stones and bedrock, and lowland riverbeds mainly consist of sand followed by mud (Table 1). Only sampling sites exhibiting reference conditions or near reference conditions were sampled (pre-classification of sites according to Moog & Sharma 2005a) (Figure 3, Figure 4). This guaranteed that substrate and current preferences of taxa were not influenced or altered due to river degradation. Altogether, 93 sampling sites in the lowland rivers and 178 sampling sites in the lower mountainous rivers were sampled (Table 1).

Figure 3: Reference site, mountain river, post-monsoon 2006. Photo: Thomas Korte.

Figure 4: Reference site, lowland river, post-monsoon 2006. Photo: Thomas Korte.

Sampling and processing

During two sampling periods (from late October 2005 to early January 2006 and from mid March 2006 to early June 2006) 271 standardized substrate specific samples were taken from different sampling teams of the ASSESS-HKH consortium (Table 2). Number of samples per river and substrate type was taken according to the relative share of the different substrate types, focusing on the dominant types. Ideally the five most abundant substrate types per country and stream type (see chapter 2.2.1) were sampled. A substrate specific sample is a single application of the net sampler with a sampling area of 25 x 25 cm and a mesh width of 500 µm.

At most sampled substrate types current velocity was measured with flow meter, and assigned to a certain current velocity class (Table 3). In Nepal and Pakistan flow meter was not available. There descriptions of flow character were used to designate current velocity class (Table 3). In addition depth and distance to shore were taken as additional environmental parameters (for manual of substrate specific sampling see Appendix 1_1).

Benthic invertebrates were identified to the lowest attainable taxonomic level. In the framework of the ASSESS-HKH project the identification work was assisted by experts who developed ”working” identification keys for the most important taxa groups; Trichoptera (Wolfram Graf, Hans Malicky), Plecoptera (Wolfram Graf, Ignac Sivec), Ephemeroptera (Thomas Soldan), Diptera (Berthold Janeĉek, Rudolf Rozkosny), Coleoptera (Manfred Jäch) and Mollusca and Oligochaeta (Hasko Nesemann). For the family Ephemerellidae (Ephemeroptera) the required identification key was compiled by the author (Appendix 1_2). Further literature used for identification is listed separately in the chapter “References”. Out of the taxonomic units of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Odonata, Diptera, Megaloptera, Lepidoptera, Gastropoda, Bivalvia and Oligochaeta, there were 68 families, 18 subfamilies, 82 genera/tribus, 5 operational taxonomic units (“in quotation marks“) and 14 species identified comprising 17,379 individuals (Table 4).

Table 1: Sampling sites characteristics. BAN = Bangladesh, BHU = Bhutan, IND = India, NEP = Nepal, PAK = Pakistan.

Ecoregion	Altitude (m)	Catchment (km²)	Hydrology	Main substrates	BAN	BHU	IND	NEP	PAK	No sampling sites	No rivers
Lowland	45-250	500-1000	permanent, pring fed	Mud, sand	11		59	23		93	21
Lower mountains	500-2500	50-500	permanent, spring fed	Large stones, bedrock		36	95	30	17	178	42
SUM					11	36	154	53	17	271	63

Table 2: Number and origin of sampled substrate types. BAN = Bangladesh, BHU = Bhutan, IND = India, NEP = Nepal, PAK = Pakistan.

Substrate type	No sampling sites	No river	Ecoregion	Country
Detritus (mostly fallen leaves)	4	2	Lowland	IND
Waterplants	10	5	Lowland	IND
Mud	20	7	Lowland	BAN, NEP
Sand	29	15	Lowland	BAN, NEP, IND
Wood	7	3	Lowland	IND
Fine gravel (0,2-2 cm)	30	15	Lowland, lower mountains	BHU, NEP, IND, PAK
Coarse gravel (2-6 cm)	37	21	Lowland, lower mountains	BHU, NEP, IND, PAK
Small stones (6-20 cm)	43	28	Lowland, lower mountains	BHU, NEP, IND, PAK
Large Stones (20-40 cm)	36	21	Lowland, lower mountains	BHU, NEP, IND, PAK
Bedrock (> 40 cm)	*55*	20	Lowland, lower mountains	BHU, NEP, IND, PAK
Sum sampled substrate types	271			

In addition to the aforementioned sampling procedure at each sampling site a Multi-Habitat sampling was carried out based on Barbour *et al.* (1999) and AQEM consortium (2002). A single Multi-Habitat sample reflects the proportion of the microhabitat types present. Investigations on Mollusca and Oligochaeta were conducted on basis of the latter sampling procedure (for further explanations see chapter 2).

1.2.2 Importance of substrate type and current velocity

Substrate type, current velocity, depth and distance from shore were analyzed in a test to explain the variability in the biotic data set. Detrended Correspondence Analysis (DCA) was used to examine the rate of turnover of benthic macroinvertebrates across the samples on the first axis of variation. With a turnover rate of 3.84, the assumption of unimodal response of species to environmental variables was made and the choice of Canonical Correspondence Analysis (CCA) to quantify the explanation shares of the environmental parameters was made. Then, partial CCA (pCCA) was used to separate and quantify the effects of each environmental parameter. Finally, a Monte Carlo permutation test revealed environmental parameters significantly explained the variability in the biotic dataset. DCA, CCA, pCCA and Monte Carlo permutation test were performed with 4.51 CANOCO for Windows (1997-2003, F. ter Braak and P. Smilauer) and 4.1 CanoDraw for Windows (1999-2003, Petr Smilauer).

Table 3: Current velocities classes.

Velocity class	Description
Class 1	No current, no visible flow, or pool, 0 cm/s
Class 2	Slow current, mostly near the shore, 1-10 cm/s
Class 3	Moderate current, 11-30 cm/s
Class 4	Distinct current, mostly accompanied with surface disturbance, 31-50 cm/s
Class 5	Fast current, surface distinctly disturbed, 51-100 cm/s
Class 6	Very fast current, broken waves at the surface, > 100 cm/s

1.2.3 Allocation of substrate and current preferences

To allocate substrate and current preferences, only taxa which were present in at least 10 samples and with at least 30 individuals were considered. Exceptions were made for three taxa occurring in less than 10 samples, but with numbers of individual greater than 30 (*Epeorus* "bispinosus", *Hydropsyche* "white stripe" and Hydroptilidae Gen. sp.). All together, 91 taxonomical units were investigated for significant substrate and current preferences (Table 4). Only selected taxa of Gastropoda, Mollusca and Oligochaeta were observed. The latter taxa originate from another data set comprising 373 sampling sites and were exclusively found on sand and/or mud. Allocation to substrate types was done in cooperation with expert Hasko Nesemann.

Table 4: Summary of all sampled taxa. In brackets number taxa finally investigated for substrate and current preferences (n = 271 samples observed). OTU = Operational taxonomic unit (see chapter 1.2.3).

Taxa	Samples	Individuals	Family	Sub-family	Genus / Tribus	OTU	Species
Ephemeroptera	190	3076	6 (6)	2 (2)	32 (16)	2 (2)	1 (0)
Plecoptera	54	363	4 (2)	1 (1)	6 (1)		
Trichoptera	172	2678	19 (12)	6 (3)	30 (9)	3 (1)	
Coleoptera	95	1185	10 (3)	2 (2)			
Diptera	153	8092	12 (7)	7 (5)	5 (4)		
Odonata	45	108	7 (2)				
Lepidoptera	8	16	1 (0)				
Megaloptera	6	7	1 (0)				
Gastropoda	64	1210	3		4		5 (5)
Bivalvia	75	559	4		5		7 (7)
Oligochaeta	10	64	1				1 (1)
SUM		17,379	68 (32)	18 (13)	82 (30)	5 (3)	14 (13)

To weight down the influence of mass occurrence in a single sample all individual numbers were log-transformed. To identify taxa significantly linked to a certain (combination of) substrate type(s), current velocity Spearman rank correlation (threshold value $r \geq 0.5$, $p \leq 0.05$) was applied for linear relationships, i.e. preference for large or small stones, and CCA (t-value biplot) for unimodal relationships. Taxa which passed the threshold criteria will be referred to as significant substrate or significant current taxa. T-value biplots were conducted with 4.1 CanoDraw for Windows (1999-2003, Petr Smilauer). Spearman rank correlation was carried out with STATISTICA 6.1 (1984-2003, StatSoft Inc.).

1.2.4 Quantification of substrate and current preferences

To describe substrate and current preferences with more detail for taxa deemed significant, a 20 point system was developed. Allocating points for substrate and current preferences to a certain taxon provides practical information for use in bioindication protocols. Taxa obtaining highest scores only in a very few substrates or current classes may be considered as good bioindicators. The allocation procedure comprised a series of steps. Numbers of individuals per sample were transformed into abundance class values. Abundance class values within each substrate type were summed. Then, points within a substrate type were converted into its relative share (%) with respect to abundance class points in all substrate classes. Finally, percentage values in each class were transformed into a numeric system distributing 20 points in total for substrate and current preferences, respectively (Figure 5).

1.2.5 Development of metrics

All taxa showing significant relation to stony substrate types were summarized to the metric “Lithal”. Taxa assigned to the metric Lithal were further subdivided into the metric “Lithobiont”, if exclusively found on stones, and to the metric “Lithophil” if significant on stones but also found on other substrate types. Taxa having a significant preference for high current velocities were combined to the metric “Lotic” (preference between current classes 3-6).

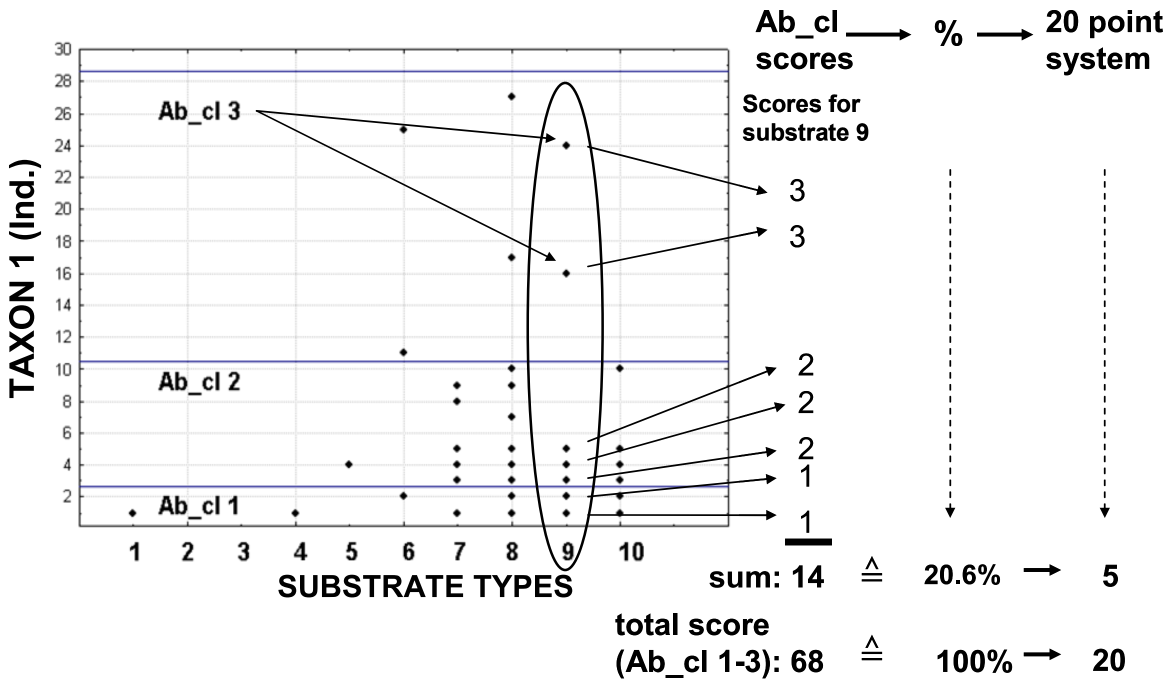


Figure 5: 20 point allocation procedure. Ab_cl = Abundance class. Substrate type 9 may stand for "large stones".

1.2.6 Ability of metrics to detect hydromorphological impact

The new metrics (Lithal, Lithobiont, Lithophil and Lotic) were tested according to their capability to detect the impact of hydromorphological degradation, namely decrease of wooded riparian vegetation, increase of bank fixation and general hydromorphological degradation reflected by PCA axis 1 values. This was restricted to the lower mountain rivers because riverbeds in the lowland consist naturally of sand and mud. Consequently, stone dwelling taxa of the latter metrics do not occur naturally in lowland rivers. The hydromorphological parameter values were derived from another data set of the same study area, and also contained biological data from 181 sampling sites. Sampling sites were divided into sites which are stressed or unstressed by the latter hydromorphological parameters. New metrics were generated for each sampling site. The generation of metrics was done on three abundance levels: (1) Individual numbers of taxa which belonged to a certain metric type were summed up; (2) Presence/absence counted only the number of taxa which were classified to a certain metric regardless of individual numbers and (3) Abundance class. Individual numbers of classified taxa were transferred into abundance class values and summed up (Table 5). Mann-Whitney U-test and Box and Whisker plots were applied on each of the three abundance levels to test the relation of the new metrics to hydromorphological stress. To evaluate the discriminatory power of the metrics, overlapping of interquartile ranges and position of medians were compared. Threshold values were defined to indicate sites which are stressed or unstressed by hydromorphological degradation. Spearman rank correlation was applied to detect intercorrelation of metrics. Box and Whisker plots were performed with STATISTICA 6.1, (1984-2003, StatSoft Inc.). Metrics were calculated with MICROSOFT OFFICE EXCEL 2003 SP3 (1985-2003, Microsoft Corporation).

Table 5: Transformation of individual numbers to abundance class values.

Abundance class	Number individuals
1	$1 \leq n < 3$
2	$3 \leq n < 11$
3	$11 \leq n < 31$
4	$31 \leq n < 101$
5	$101 \leq n < 301$
6	$301 \leq n < 1001$
7	$1001 \leq n$

Figure 6 explains the procedure for the allocation of substrate and current preferences to benthic macroinvertebrates in the rivers of the Hindu Kush-Himalaya region.

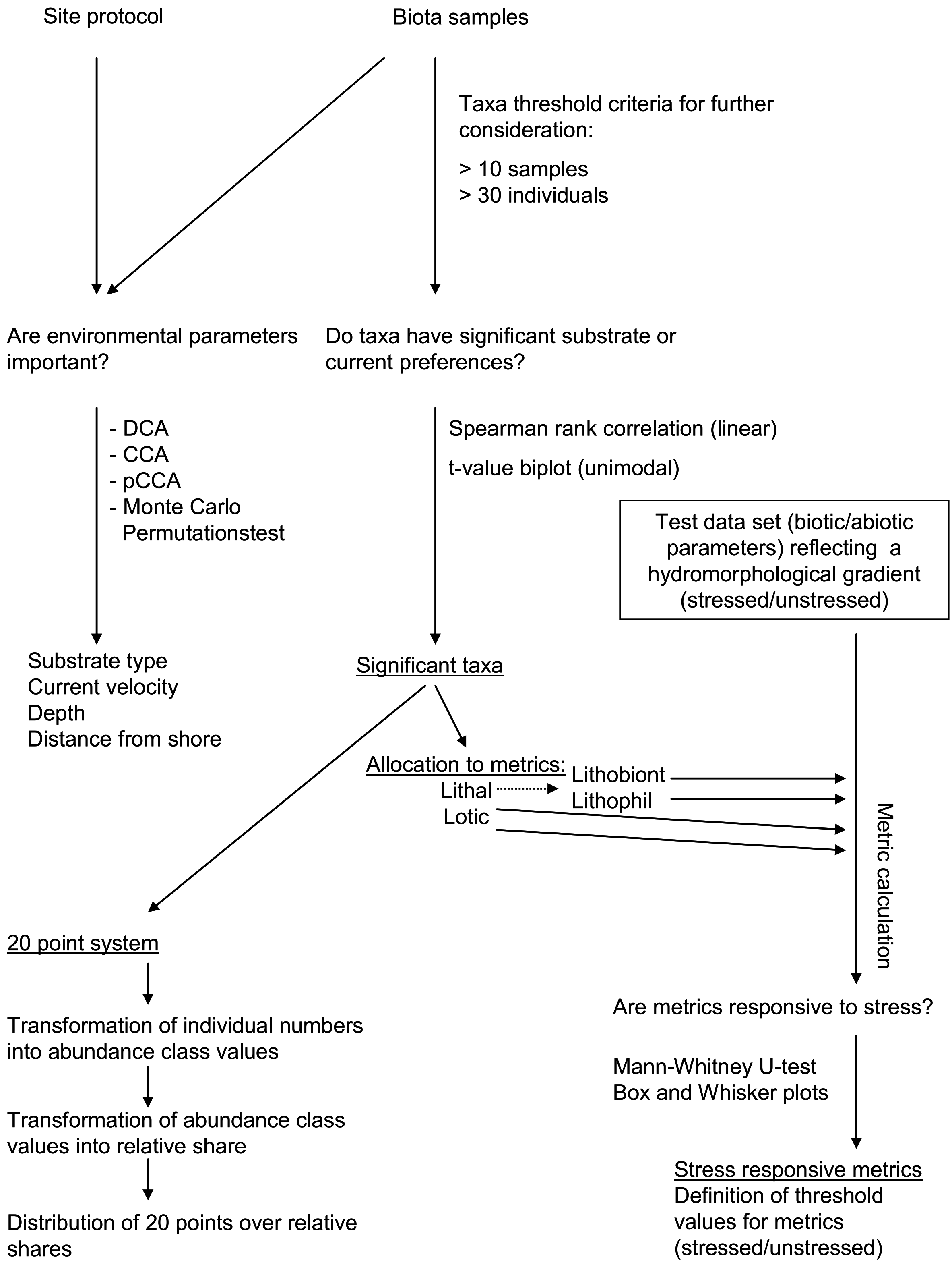


Figure 6: Procedure for the allocation of substrate and current preferences to benthic macroinvertebrates and usage as bioindicators. Simplified.

1.3 Results

1.3.1 Importance of substrate type and current velocity for community composition

Canonical Correspondence Analysis explains 27.6 % of variance within the data set (cumulative percentage variance of species data, first four CCA axes). The variance explained by the CCA is best reflected by the substrate types (Figure 7). The most important gradient is the substrate “mud”, which is closely correlated to the first CCA axis. The second CCA axis is mainly a substrate gradient from bedrock/large stones to sandy substrate types. Environmental parameters distance from shore and depth exhibit small arrows, hence being of minor importance (Figure 7). The different eigen-values of the pCCA revealed the distribution of taxa being highly dependent on sub-strate types compared to the other observed environmental parameters (Table 6). However, the Monte Carlo permutation test revealed that the distribution of taxa proximal to bedrock, large stones, coarse gravel, fine gravel, sand, and mud is also significantly correlated with current velocity, distance from shore and depth (Table 7).

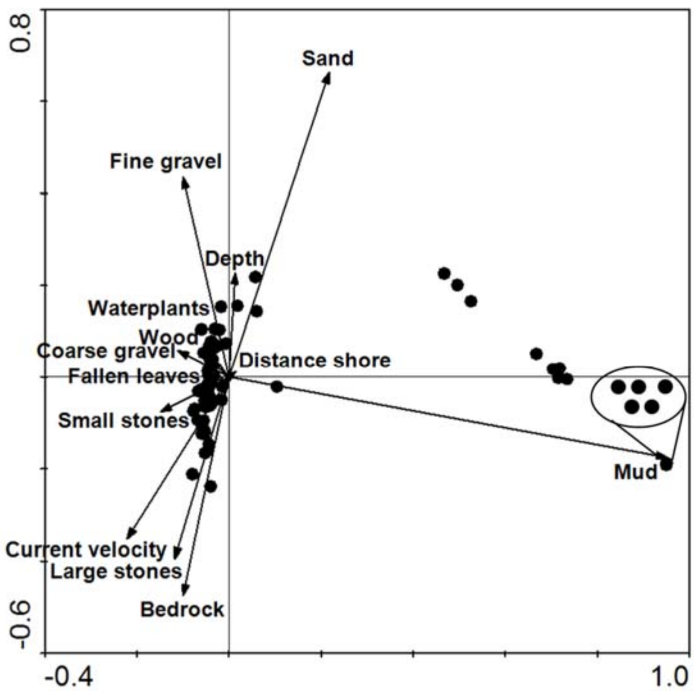


Figure 7: CCA biplot. Environmental parameters (arrows) and taxa (points).

Table 6: pCCA-results. Sum of all canonical eigenvalues per environmental variable.

Parameter	Sum eigenvalues
Substrate	0.959
Flow velocity	0.039
Depth	0.034
Distance shoreline	0.028

Table 7: Results Monte Carlo permutations test.

Environmental variable	p-value
Bedrock	0.002
Large stones	0.002
Coarse gravel	0.002
Current velocity	0.002
Distance shore	0.008
Fine gravel	0.014
Sand	0.014
Depth	0.028
Mud	0.042

1.3.2 Allocation of substrate and current preferences

50 taxa were detected with significant preferences to certain substrate types (Figure 8, Table 8) and/or current velocities (Figure 9, Table 8). 15 taxa show significant preference both for certain substrate type and certain current velocity. 34 insect taxa were detected with significant preference to stony substrates of these 14 Ephemeroptera taxa, 14 Trichoptera taxa, two Coleoptera taxa, one Plecoptera taxon and one Odonata taxon. *Baetiella* sp., Simuliidae Gen. sp. and Orthocladiinae_Diamesinae Gen. sp. also significantly prefer other but mineral substrate types. 12 Mollusca taxa significantly indicate sand and mud. *Corbicula striatella* DESHAYES (Bivalvia) and *Thiara lineata* GRAY (Gastropoda) additionally exhibit significant preference for sand. 12 taxa were detected with significant preferences for faster current velocities comprising eight Trichoptera taxa, two Ephemeroptera taxa, and one Diptera taxon. Seven taxa show preferences for fast and slow current velocities (indifferent).

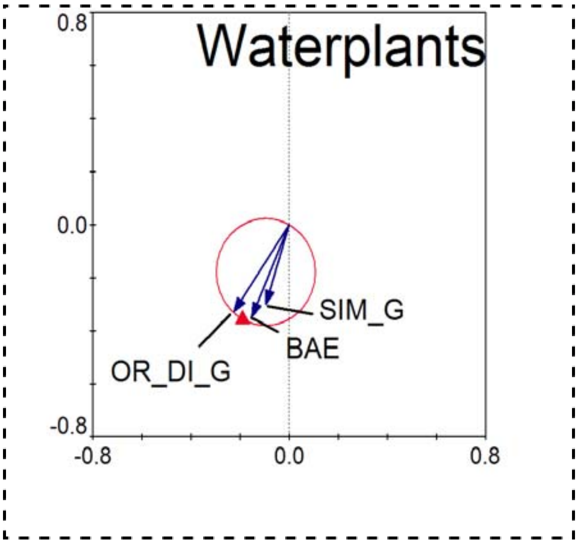


Figure 8a

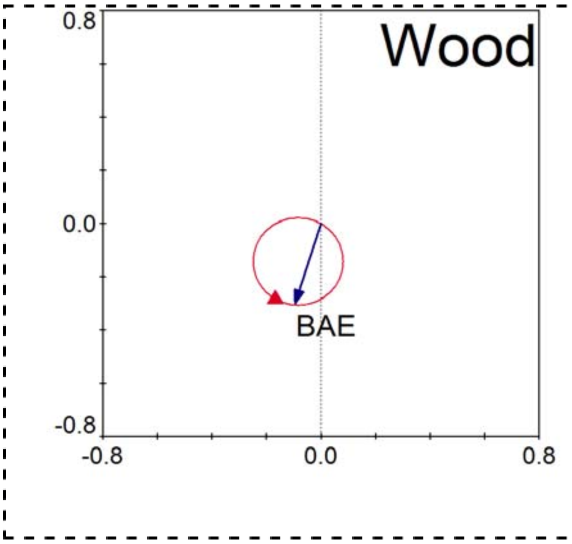


Figure 8b

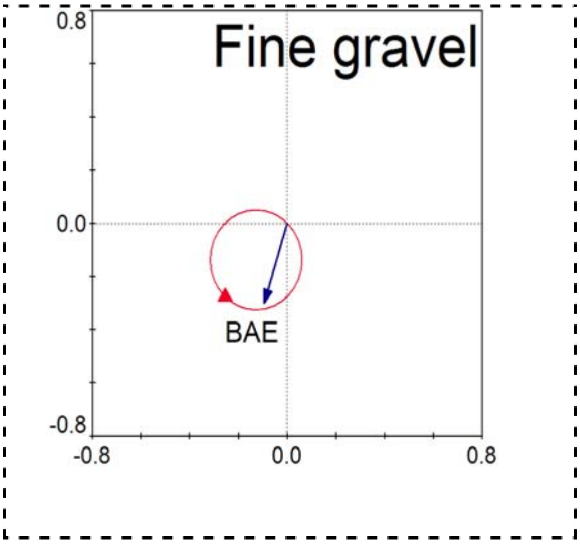


Figure 8c

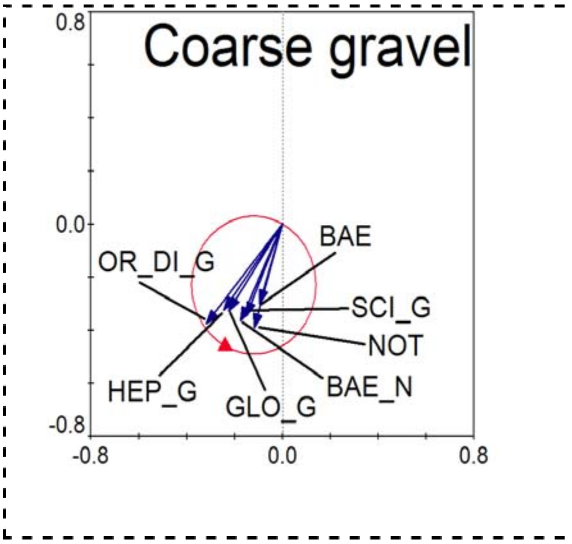


Figure 8d

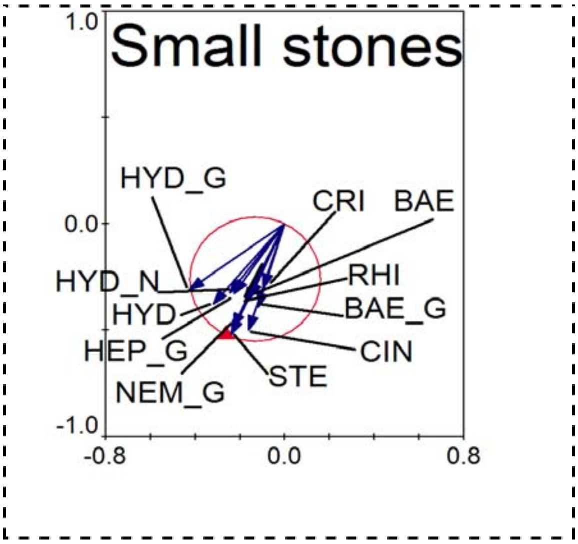


Figure 8e

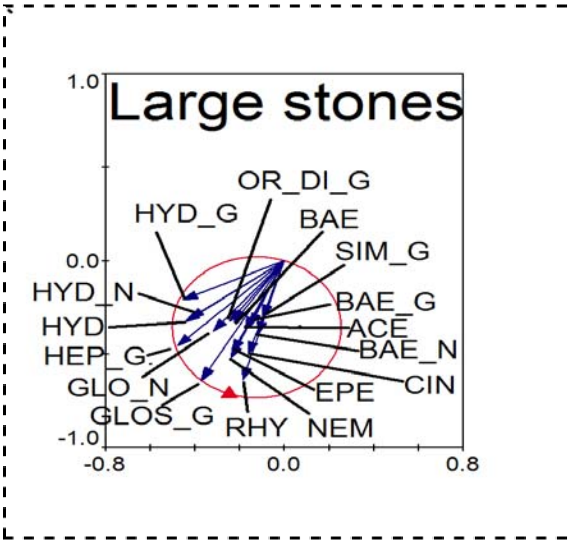


Figure 8f

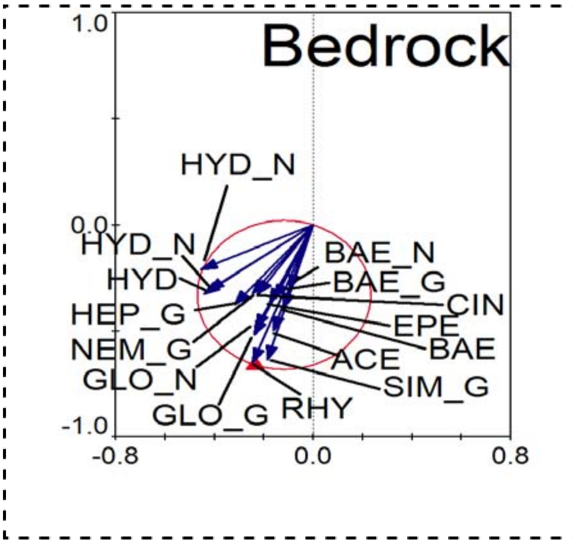


Figure 8g

Figure 8 a-g: T-value biplots substrate. Symbol indicates substrate type. Arrows indicate species. Species lines that end in the circle have a positive regression coefficient for that environmental variable with corresponding t-value > 2.0. Abbrevations: ACE = *Acentrella* sp., AGA_N = Agapetinae Gen. sp., ATH_G = Athericidae Gen. sp., BAE = *Baetiella* sp., BAE_G = Baetidae Gen. sp., BAE_N = Baetinae Gen. sp., CHE = *Cheumatopsyche* sp., CIN = *Cincticostella* sp., CRI = *Crinitella* sp., EPE = *Epeorus* sp., GLO_G = Glossosomatidae Gen. sp., GLO_N = Glossosomatinae Gen. sp., HEP_G = Heptageniidae Gen sp., HYD = *Hydropsyche* sp., HYD_G = Hydropsychidae Gen. sp., HYD_N = Hydropsychinae Gen. sp., NEM_G = Nemouridae Gen. sp., NOT = *Notacanthurus* sp., OR_DI_G = Orthocladiinae_Diamesinae Gen. sp., RHI = *Rhithrogena* sp., RHY = *Rhyacophila* sp., SCI_G = Scirtidae Gen. sp., SIM_G = Simuliidae Gen. sp., STE = *Stenopsyche* sp.

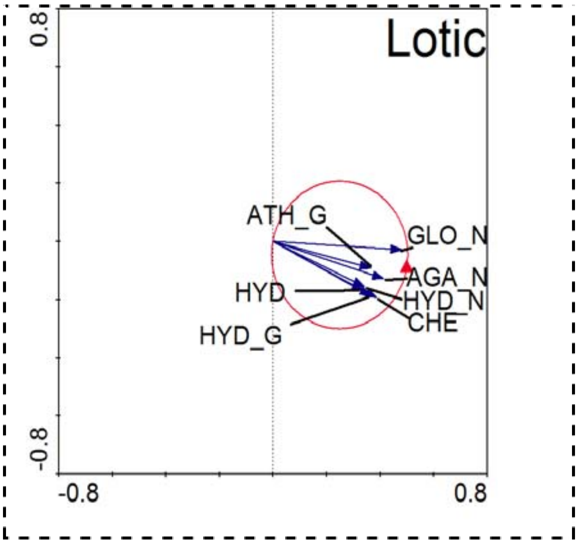


Figure 9: T-value biplots lotic (current velocity classes 3-6). Symbol indicates current. Arrows indicate species. Species lines that end in the circle have a positive regression coefficient for that environmental variable with corresponding t-value > 2.0. Abbreviations: AGA_N = Agapetinae Gen. sp., ATH_G = Athericidae Gen. sp., CHE = *Cheumatopsyche* sp., GLO_N = Glossosomatinae Gen. sp., HYD = *Hydropsyche* sp., HYD_G = Hydropsychidae Gen. sp., HYD_N = Hydropsychinae Gen. sp.

Table 8: Summary Spearman rank coefficients. Only significant preferences p-level < 0.05.

Taxa group	Taxon	Stony substrate types	Current, Lotic	Current, Indifferent
EPH	*Acentrella* sp.	0.5		
EPH	*Baetiella* sp.	0.56		
EPH	*Choroterpes* sp.	0.65		
EPH	*Cincticostella* sp.	0.67		
EPH	*Cinygmina* sp.	0.63		
EPH	*Crinitella* sp.	0.55		
EPH	*Drunella* sp.	0.63	0.57	
EPH	*Epeorus* sp.	0.66		0.55
EPH	*Epeorus* "bispinosus"	0.62		
EPH	*Ephemera* sp.			0.7
EPH	*Notacanthurus* sp.	0.62		0.6
EPH	*Rhithrogena* sp.	0.63	0.51	
PLE	Nemouridae Gen. sp.	0.55	0.55	
TRI	Agapetinae Gen. sp.	0.67		
TRI	Brachycentridae Gen sp.	0.8		
TRI	*Cheumatopsyche* sp.	0.6	0.5	
TRI	Glossosomatidae Gen. sp.	0.62		
TRI	Glossosomatinae Gen. sp.	0.61	0.52	
TRI	*Goera* sp.	0.6	0.55	
TRI	*Hydropsyche* "white stripe"	0.53		0.68
TRI	Hydroptilidae Gen. sp.		0.81	
TRI	*Rhyacophila* sp.	0.7	0.51	
TRI	*Setodes* sp.	0.72		
TRI	*Stenopsyche* sp.	0.67		
TRI	*Uenoa* sp.	0.87		
COL	Eubrianacinae Gen. sp.	0.68		
COL	Scirtidae Gen. sp.	0.61		0.5
DIP	Athericidae Gen. sp.			0.55
ODO	Gomphidae Gen. sp.	0.52		

1.3.3 Quantifying substrate and current preferences

44% (15 taxa) of significant substrate taxa receive highest scores for substrate type “small stones”, although the share of “small stones” is only 15% of all sampled substrate types (Table 9, Appendix 1_3, Table 2). Including taxa with equal scores for “small stones” and other lithal fractions 59% (20) of taxa most prefer “small stones” (Table 9, Appendix 1_3). Of these, 11 taxa show clear unimodal response over the size range of stony substrate types with optimum in “small stones” (Appendix 1_3). Five taxa preferred “large stones” (two) and “bedrock” (three) of which *Uenoa* sp., *Cincticostella* sp. and Brachycentridae Gen. sp. were almost only found on “large stones” and “bedrock” (Table 9; Appendix 1_3). Four taxa receive 10 points or more in one substrate type, and additionally occur only in three or fewer substrate types. In addition to *Uenoa* sp. these were *Epeorus* „bispinosus”*, Epeorus* sp.*,* and *Rhithrogena* sp. (Table 9; Appendix 1_3).

Eight “significant” current taxa show clear unimodal response over range of current velocities with maximum scores for “moderate” or “distinct current”. Altogether, 47% of taxa (eight) receive highest scores for “moderate current”. None of the observed taxa most prefer “very fast current” (Table 10, Appendix 1_4). The highest scores for slow current preference are assigned to *Ephemera* sp., *Cinygmina* sp. and *Hydropsyche* “white stripe” (Table 10, Appendix 1_4). *Ephemera* sp. was found in only three current classes exclusively, and additionally 10 points were assigned for current class “no current” (Table 10, Appendix 1_4). Only *Drunella* sp. and Hydroptilidae Gen. sp. most prefer “fast current”. However, *Drunella* sp. regularly occurs in all current classes (Table 10, Appendix 1_4). But, Hydroptilidae Gen. sp. were found in only three lotic classes and additionally receive more than 10 points for current classified as “fast” (Table 10, Appendix 1_4).

1.3.4 Development of metrics

34 insect taxa show significant preferences for stony substrate types, and were combined to metric Lithal. Of these 13 taxa were exclusively found on stony substrate types. The latter built the metric Lithobiont and consists of seven Trichoptera taxa and six Ephemeroptera taxa. 21 taxa were assigned to the metric Lithophil comprising eight Ephemeroptera taxa, seven Trichoptera taxa, one Plecoptera, two Coleoptera, two Diptera, and one Odonata taxon (Table 9).

11 taxa show significant preferences for fast current velocities. These were combined to the metric Lotic comprising eight Trichoptera taxa, two taxa of Ephemeroptera, and one Diptera taxon. No taxon was detected with preferences to slow current velocities (Lentic). Seven taxa show preferences for both slow to moderate current velocities (Indifferent) comprising four Ephemeroptera, one Plecoptera, one Trichoptera and one Coleoptera taxa (Table 10).

Table 9: Substrate preferences. Metric assignment and 20 point allocation. x indicates significant preference detected by Spearman rank correlation or t-value biplot. For comparison see also Appendix 1_3.

Taxa group	Taxon	No samples	No individuals	Lithal	Lithophil	Lithobiont	Fallen leaves	Waterplants	Wood	Mud	Sand	Fine gravel	Coarse gravel	Small stones	Large stones	Bedrock
EPH	*Acentrella* sp.	47	193	x	x								2	3	3	12
EPH	Baetinae Gen. sp.	128	1678	x	x			2	1		1	3	3	3	3	4
EPH	Baetidae Gen. sp.	143	1811	x	x			1	1	1	1	3	3	3	3	4
EPH	*Baetiella* sp.	35	189	x	x			1					2	2	4	11
EPH	*Choroterpes* sp.	15	103	x	x			1			1	3	6	7	1	1
EPH	*Cincticostella* sp.	19	77	x		x							3	3	13	1
EPH	*Cinygmina* sp.	24	59	x	x							2	3	5	5	5
EPH	*Crinitella* sp.	12	55	x		x						3	3	8	3	3
EPH	*Drunella* sp.	10	443	x		x							4	8	8	
EPH	*Epeorus* sp.	23	78	x		x								12	5	3
EPH	*Epeorus* "bispinosus"	7	48	x		x								12	8	
EPH	Heptageniidae Gen. sp.	61	327	x	x							2	4	8	4	2
EPH	*Notacanthurus* sp.	12	46	x	x		1					3	3	7	6	
EPH	*Rhithrogena* sp.	10	64	x		x							10	10		
PLE	Nemouridae Gen. sp.	11	104	x	x		2						4	5	9	
TRI	Agapetinae Gen. sp.	13	32	x		x						2	4	6	4	4
TRI	Brachycentridae Gen sp.	10	326	x	x								1	2	16	1
TRI	*Cheumatopsyche* sp.	38	223	x	x		1		2			2	3	8	4	
TRI	Glossosomatidae Gen. sp.	29	97	x		x						1	5	7	4	3
TRI	Glossosomatinae Gen. sp.	19	64	x		x						1	5	7	5	2
TRI	*Goera* sp.	10	32	x	x			1				11	5	3		
TRI	*Hydropsyche* "white stripe"	9	32	x	x			1				3	3	13		
TRI	*Hydropsyche* sp.	69	478	x	x			2				2	2	9	4	1
TRI	Hydropsychidae Gen. sp.	95	855	x	x			2	1			2	3	8	3	1
TRI	Hydropsychinae Gen. sp.	89	789	x	x			2	1			2	3	8	3	1
TRI	*Rhyacophila* sp.	24	35	x		x							2	6	6	6
TRI	*Setodes* sp.	15	39	x		x						2	7	7	4	
TRI	*Stenopsyche* sp.	20	47	x		x						1	3	10	6	
TRI	*Uenoa* sp.	10	91	x		x								1	6	13
COL	Eubrianacinae Gen. sp.	10	18	x	x				1		1	4	5	5	4	
COL	Scirtidae Gen. sp.	11	58	x	x			1				1	8	6	3	1
DIP	Orthocladiinae_ Diamesinae Gen. sp.	91	3377	x	x			1	1		1	3	3	3	3	5
DIP	Simuliidae Gen. sp.	55	854	x	x			2						6	6	6
ODO	Gomphidae Gen. sp.	32	59	x	x			1			1	6	6	4	1	1

Table 10: Current preferences. Metric assignment and 20 point allocation. x indicates significant preference detected by Spearman rank correlation or t-value biplot. For comparison see also Appendix 1_4.

Taxa group	Taxon	No samples	No individuals	Lotic	Indifferent	Class 1 (Lentic)	Class 2 (Lentic)	Class 3 (Lotic)	Class 4 (Lotic)	Class 5 (Lotic)	Class 6 (Lotic)
EPH	*Rhithrogena* sp.	10	64	x				5	5	5	5
EPH	*Drunella* sp.	443	10	x		2	2	4	4	6	2
EPH	*Ephemera* sp.	12	38		x	10	6	4			
EPH	*Epeorus* sp.	23	78		x		2	6	8	2	2
EPH	*Notacanthurus* sp.	12	46		x	2	5	5	7	1	
EPH	*Cinygmina* sp.	24	59		x	8	6	2	2	2	
PLE	Nemouridae Gen. sp.	11	104		x	2	2	6	6	2	2
TRI	Hydroptilidae Gen. sp.	6	54	x					4	14	2
TRI	*Hydropsyche* sp.	69	478	x		2	5	8	3	1	1
TRI	Cheumatopsyche sp.	38	223	x		3	5	5	4	3	
TRI	Hydropsychinae Gen. sp.	89	789	x		2	4	8	4	1	1
TRI	*Goera* sp.	10	32	x		1	4	7	4	4	
TRI	*Rhyacophila* sp.	24	35	x				9	6	3	2
TRI	Glossosomatinae Gen. sp.	19	64	x			1	7	6	3	3
TRI	Agapetinae Gen. sp.	13	32	x			2	8	5	3	2
COL	Scirtidae Gen. sp.	11	58		x	1	5	6	5	2	1
DIP	Athericidae Gen. sp.	12	39	x		1	5	9	4	1	

1.3.5 Ability of new metrics to detect impacts of hydromorphological degradation

The metrics Lithal, Lithophil and Lotic are decisively (all $p < 0.001$) able to detect the three observed hydromorphological stressors regardless of abundance level (Table 11). The metric Lithobiont demonstrates significant discriminatory power on abundance class level and on individual number level. Box and Whisker plots (abundance class level) also show ability of all metrics to detect hydromorphological degradation. The metrics Lotic, Lithal and Lithophil reveal the best discriminatory power exhibiting both medians outside the interquartile range overlap (Figure 10). The metric Lithobiont has at least one median inside the interquartile range overlap. However, no metric is able to distinguish clearly between stressed and unstressed sites as all interquartile ranges are overlapping (Figure 10). Threshold values for stressed or unstressed sites were defined on basis of Box and Whisker plots. Each of stressed and unstressed sites the mean of the medians per metric over all of the three observed hydromorphological stressors seems suitable (Table 12). Spearman rank correlation revealed that all of the four metrics are inter-correlating (Spearman r > 0.5, p < 0.05).

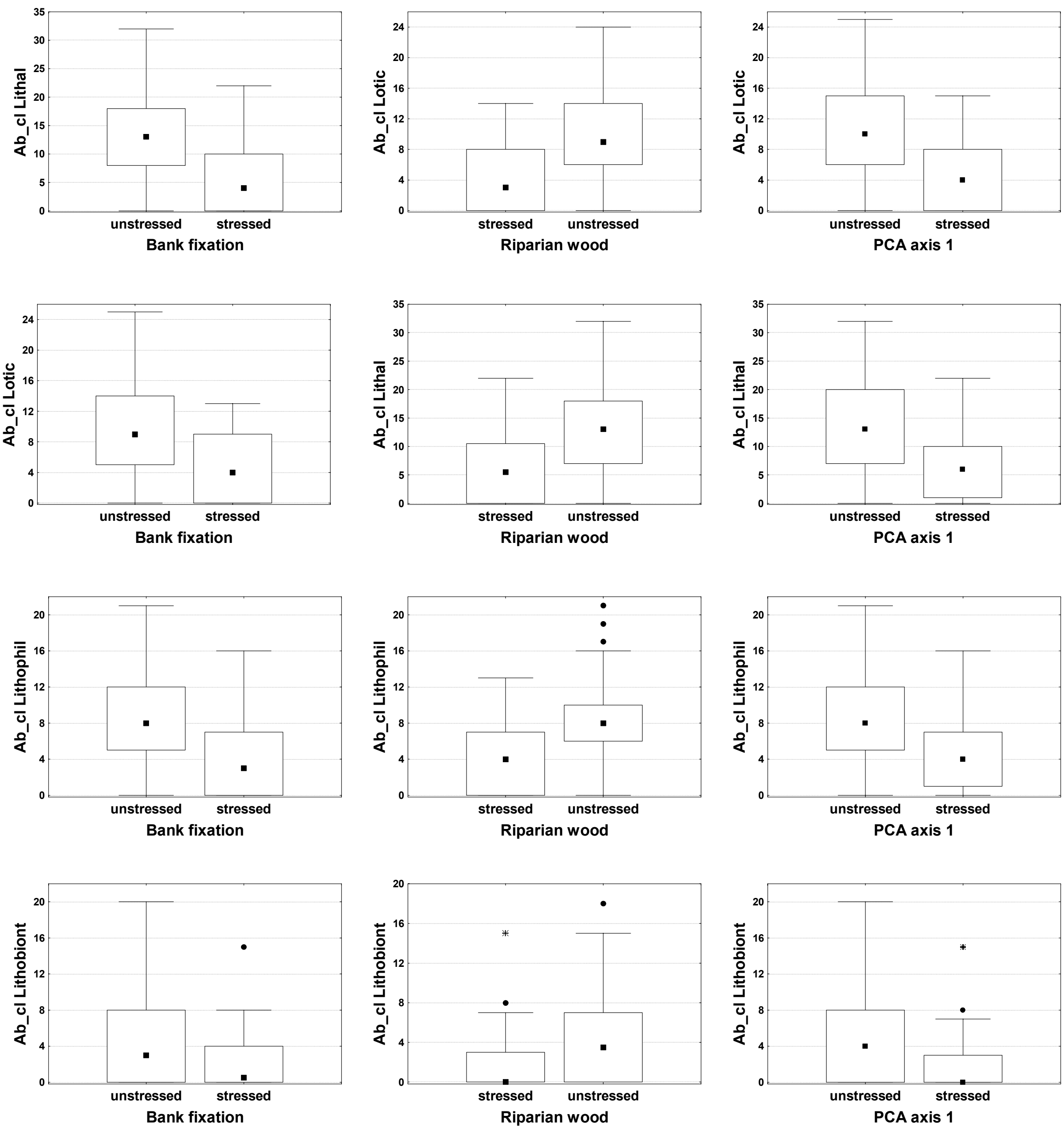


Figure 10: New metrics vs. different hydromorphological stressors. n = 181 sampling sites. Box = 25%-75% Interquartile, Whiskers = Non-Outlier Range, Squares = Median, Circle = Outlier, Cross = Extremes. Ab_cl = Abundance class.

Table 11: p-level results of Mann-Whitney U-test. Comparison between sampling sites stressed or unstressed under different hydromorphological stressors and new metrics. Ab_cl = abundance classes. n.s. = not significant. n = 181 sampling sites.

Metric	PCA axis 1	% riparian wooded vegetation	% bank fixation
No taxa Lotic	< 0.001	< 0.001	< 0.001
Ab_cl Lotic	< 0.001	< 0.001	< 0.001
No individuals Lotic	< 0.001	< 0.001	< 0.001
No taxa Lithal	< 0.001	< 0.001	< 0.001
Ab_cl Lithal	< 0.001	< 0.001	< 0.001
No individuals Lithal	< 0.001	< 0.001	< 0.001
No taxa Lithophil	< 0.001	< 0.001	< 0.001
Ab_cl Lithophil	< 0.001	< 0.001	< 0.001
No individuals Lithophil	< 0.001	< 0.001	< 0.001
No taxa Lithobiont	n.s.	< 0.05	n.s.
Ab_cl Lithobiont	< 0.001	< 0.001	< 0.01
No individuals Lithobiont	< 0.001	< 0.01	< 0.01

Table 12: Threshold values of metrics. Mean of median per metric type over all observed hydromorphological parameters.

Metric	Threshold unstressed	Threshold stressed
Lotic	> 9	< 4
Lithal	> 13	< 6
Lithobiont	> 8	< 4

1.4 Discussion

1.4.1 Importance of substrate and current velocity for aquatic macroinvertebrates

Of the observed environmental parameters substrate type and current velocity are the most important for benthic invertebrates. CCA explains 27.6 % of total variance in the data set. The observed parameters explain a significant share of the variability within the data set and are demonstrably of importance for the distribution of benthic invertebrates. Nevertheless, other environmental parameters must be important for the distribution of taxa, besides the observed, though these results are consistent with many other studies showing that substrate type and current velocity primarily rule the distribution of taxa in the river bed (Cummins & Lauf 1968, Minshall & Minshall 1977, Statzner *et al.* 1988, Rempel *et al.* 2000, Buss *et al.* 2004). Other natural factors influencing the distribution of taxa are composition of substrate types (Gurtz & Wallace 1984), physical attributes of substrate types (Brooks *et al.* 2005), hydraulic conditions (Statzner *et al.* 1988), flow refugia availability (Gjerløv *et al.* 2003), disturbance (Resh *et al.* 1988, Leopold 1994), light and colour (Robinson & Minshall 1986, Casey & Clifford 1989), depth (Brooks *et al.* 2005), temperature and season (Bournaud *et al.* 1987), biological interactions (Lancaster *et al.* 1990, Thomson 2002), resource availability (Richardson 1993) and the biological state of a taxa's life cycle (Minshall 1984). In addition, inter-correlation between environmental parameters must be considered in order to evaluate the importance of a single parameter for the biota. Consequently subtle interferences of environmental parameters result in temporal and spatial heterogeneity of benthic invertebrate distribution.

1.4.2 Allocation of substrate and current preferences

Significant substrate and current preferences were detected for 50 taxa of benthic invertebrates. The findings were compared against suitable literature originating from the biogeographic regions categorized as: Oriental (Ulmer 1955, 1957, Stauder 1999, Dudgeon 1999, Yule & Sen 2004, Nesemann 2007), the West-Palearctic (Eurolimpacs consortium 2008), Nearctic (Stewart & Stark 1993, Wiggins 1996, Merrit & Cummins 1996), and East-Palearctic (Lepneva 1964) (Appendix 1_5). The data are the first about substrate and/or current preferences for *Baetiella* sp., *Cincticostella* sp., *Crinitella* sp., *Drunella* sp. and *Cinygmina* sp. Literature showed additional substrate preferences only for *Drunella* sp., Brachycentridae Gen. sp., *Rhithrogena* sp. and *Setodes* sp. Two taxa were assigned to additional velocity classes (*Rhithrogena* sp. and Hydroptilidae Gen. sp.). Additional information in the consulted literature shows that species of the investigated genera and families have a broader range of substrate and current preference than at the taxa level observed in this study. Therefore, the findings are only applicable in the rivers of the lower mountains of the Hindu Kush-Himalaya region. Nevertheless, the introduced sampling procedure and the data analysis used provide consistent information about substrate and current preferences, and may be used in regions of the world where literature knowledge is scarce.

1.4.3 Quantifying substrate and current preferences

Most substrate taxa prefer small stones (6-20 cm) as life space. Beauger *et al* (2006) observed highest benthic invertebrate diversity in river Allier (France) in comparable substrate sizes (3-25 cm). Hawkins (1984) assumed that small stones are complex habitats and share traits in common with most other substrate types. The top of small stones are comparable with the top of bedrock and large boulders, providing also habitat for grazers of algae and mosses. Sand, gravel and detritus deposit in the interstitial spaces around small stones, providing associate life space and food. In addition, interstitial space is easily accessible and provides shelter. To summarize, small stones have proven to be the most utilizable habitat and, consequently, sustainable river management should contain specific measures for the conservation of what is characteristic in this study of a small stones environment.

Those taxa investigated that predominately occur on “large stones” and “bedrock” are three in number, *Uenoa* sp., *Cincticostella* sp., and Brachycentridae Gen. sp. Each of Ephemeroptera taxa *Epeorus* sp., *Epeorus* „bispinosus”, *Drunella* sp. and *Rhithrogena* sp. were exclusively found on three substrate types with highest scores for “small stones”. Literature gives indifferent substrate preferences on family level for Brachycentridae Gen. sp. (Graf *et al.* 2006, information for Europe). No information about substrate preferences is available for *Uenoa* sp. and *Cincticostella* sp. Buffagni *et al.* (2007, information for Europe) and Yule & Sen (2004, information for Malaysian region) allocate *Epeorus* sp. and *Epeorus* „bispinosus“ to stony substrates. In Europe, species of *Drunella* sp. live on stones, but also on waterplants (Buffagni *et al.* 2007). *Rhithrogena* sp. is not treated commonly in literature. In India Stauder (1999) found the genus only at stones, while in Europe it is found on substrates other than stony substrate types (Buffagni *et al.* 2007). With respect to the literature, the findings may be interpreted as follows. All of the previously mentioned seven taxa may serve as good bioindicators for the observed region. Their ecological potential toward habitat alteration is small, because they prefer only a few substrate types (“small stones”, “large stones” and “bedrock”). Consequently, impacts on these substrates and its connected parameters, e.g. through sedimentation may not be compensated by the latter taxa through evading to other life spaces.

Most significant current taxa prefer “moderate” or “distinct” velocities (11-50 cm/s). Other investigations also revealed comparable current preference for many taxa (Schmedtje 1995). In terms of rivers stressed by damming, the taxa preferring “moderate” or “distinct” velocities, and exhibiting low ecological potential with respect to current velocity may vanish from the ecosystem. Within this investigation the metric Lotic was generated comprising taxa preferring “moderate” to “distinct” current velocities. For future investigations metric Lotic may be tested, where suitable, to detect impacts of impoundment and damming in the Hindu Kush-Himalaya region.

1.4.4 Development of metrics

Four metrics were developed for usage in river assessment. The metric Lithobiont is built of 13 taxa found exclusively on stones. 21 taxa are combined to form metric Lithophil. The latter taxa exhibit significant preferences for stony substrates, but can be found on other substrate types. The metric Lotic consists of 11 taxa with significant preferences for moderate to faster current velocities. Schmedtje & Colling (1996) classified substrate and current preferences of more than 1500 aquatic taxa mostly based on literature research. Substrate and current preferences were combined to metrics containing taxa sharing the same information. These metrics are suitable for ecological river assessment (Meier *et al.* 2006). Also, in other parts of the world metrics are the basis for describing and evaluating ecological processes (Barbour *et al.* 1999, Hering *et al.* 2004b, Baptista *et al.* 2007). The new metrics combine taxa that show preferences for stony substrates and faster current velocities, and because the rivers of the Hindu Kush-Himalaya region are stressed by several hydromorphological impacts which lead to habitat alteration these metrics may serve as indicators to describe and assess the impact.

1.4.5 Ability of metrics to detect impacts of hydromorphological degradation

Metrics Lotic, Lithal and Lithophil exhibit best the ability to detect the impact of hydromorphological degradation in rivers of the lower mountains in the Hindu Kush-Himalaya region (Figure 10). Threshold values defined for each of the metrics indicate stressed or unstressed sites. Other investigations also showed that the knowledge about substrate and current preferences of taxa may be used to detect the impact of hydromorphological degradation (Lorenz *et al.* 2004, Schmedtje 1995). Meier *et al.* (2006) applied metrics based on substrate and current preference of taxa to develop a multimetric index for Germany. In the rivers of the Hindu Kush-Himalaya region removal of mineral riverbed material, damming for irrigation, and cutting of wooded riparian vegetation are common stressors which lead to loss of habitat for benthic invertebrates. The new metrics are able to detect the impact by decreasing values. Since of all of the new metrics are inter-correlating only one of the three metrics needs to be applied for assessment of hydromorphological impact. In addition, the application should be carried out on the abundance class values of taxa. This lowers the influence of mass occurrence of taxa and guarantees a sufficient value range.

2 Development of a Multimetric Index for Ecological River Quality Assessment

2.1 Introduction

In many parts of the world benthic macroinvertebrates are used for ecological river assessment. Among a variety of potentially suited assessment methodologies multi-metric indices are increasingly popular, particularly in North America (e.g. Barbour *et al.* 1996, Maxted *et al.* 2000, Weigel *et al.* 2002), South America (Baptista *et al.* 2007, Moya *et al.* 2007, Silveira *et al.* 2005), Europe (e.g. Böhmer *et al.* 2004, Hering *et al.* 2004a, Morais *et al.* 2004, Ofenböck *et al.* 2004, Vlek *et al.* 2004) and South-Africa (Ollis *et al.* 2006). In Asia (Oriental biogeographic region) river assessment with benthic macroinvertebrates is still in its infancies. The knowledge about riverine biodiversity is incomplete and biomonitoring and conservation of rivers is underdeveloped (Dudgeon 2003). In the Hindu Kush-Himalayan region, targeted by our study, first steps towards biological river quality assessment with benthic macroinvertebrates were previously done in Nepal (Sharma & Moog 2005, Nesemann *et al.* 2007) and India (Central Pollution Control Board 1999), applying modified versions of the Biological Monitoring Working Party (BMWP) and the Average Score Per Taxon (APST) (Armitage *et al.* 1983), respectively. However, assessment methods based on benthic macroinvertebrates are potentially well-suited tools for water management in Asia, due to steep pollution gradients and the impacts of other stressors (e.g. Messerli & Ives 1997, Merz *et al.* 2003, Central Pollution Control Board 2003a, 2003b, Sharma *et al.* 2005, Pradhan *et al.* 2005), which are well reflected by the biota. Despite the widespread lack of invertebrate-based assessment methods in the Hindu Kush-Himalayan region several studies deal with other aspects of the benthic invertebrate fauna, e.g. structural and functional aspects (Meren *et al.* 1984, Chowdhary 1984, Sharma 1986, Kumar 1987, Ahmad & Singh 1989, Singh & Srivastava 1989, Mohan *et al.* 1989, Negi & Singh 1990, Gupta & Michael 1992, Dobriyal *et al.* 1992, Rundle *et al.* 1993, Ormerod *et al.* 1994, Suren 1994, Brewin *et al.* 1996, Brewin *e al.* 2000, Nesemann *et al.* 2007).

This part of the thesis aims at developing a multimetric assessment procedure for streams in the low mountain areas of the Hindu Kush-Himalayan region and covered the countries Pakistan, India, Nepal, Bhutan and Bangladesh. The method is designed for use in practical water management. The development procedure included the delineation of stream types mainly based on the ecoregion approach, the a-priori classification of sampling sites to cover a gradient in environmental quality, and included the correlation of a large number of biotic indices against environmental parameters. I tested if metrics frequently applied in North America, Europe and South America are also suited to detect perturbation of rivers in the Hindu Kush-Himalaya region and if metrics are stressors-specific, reflecting e.g. the degree of organic pollution, eutrophication, land-use of the catchment or hydromorphological degradation. Finally, for each of the stream types a set of robust core metrics reflecting river degradation was selected and combined into a multimetric system for integrative assessment of river ecosystems.

2.2 Material and Methods

The study area has been previously described (Chapter 1.2) and information will only focus on new information here.

2.2.1 Study area, site selection and sampling

Study area

Multimetric indices were developed for five stream types, i.e. streams within the same ecoregion and with a comparable size. Stream types were defined based on environmental parameters and not by the invertebrate assemblage, as many of the sampling sites are heavily impacted and their invertebrate assemblage is thus determined by perturbation rather than by natural parameters. Ecoregions (based on the WWF Global 2000 ecoregions, Olson *et al.* 2001), catchment size and altitude were used to define five stream types (Figure 11, Table 13). All stream types investigated are characterized by permanent flow, fed by sources and of small to medium size (i.e. wadeable in the dry season) (Moog & Sharma 2005b). In the following, ecoregion names are used as synonyms for the stream types.

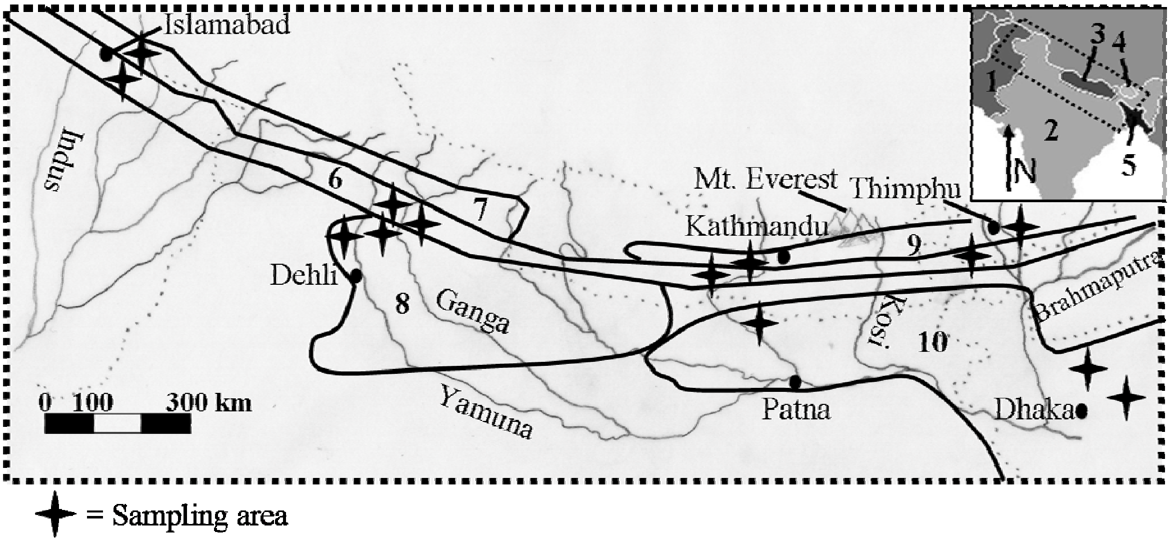


Figure 11: Study area. Ecoregions observed. 1 = Pakistan; 2 = India; 3 = Nepal; 4 = Bhutan; 5 = Bangladesh; 6 = Himalayan Subtropical Pine Forest; 7 =Western Himalayan Broadleaf Forest; 8 = Upper Gangetic Plains; 9 = Eastern Himalayan Broadleaf Forest; 10 = Lower Gangetic Plains.

Table 13: Number sampling sites per stream type and country.

Ecoregion	Altitude range (m)	Catchment size (km²)	Bangladesh	Bhutan	Nepal	India	Pakistan	No sites
Eastern Himalayan Broadleaf Forest	1000-2500	50-500		17	17			34
Himalayan Subtropical Pine Forest	500-2000	50-500		17	17	19	25	78
Western Himalayan Broadleaf Forest	1000-2500	50-500					17	17
Lower Gangetic Plains	45-250	500-1000	34		17			51
Upper Gangetic Plains	45-250	500-1000				18		18

Site selection

In each country sharing a stream type sites of different environmental quality were selected, in most cases equally distributed over a five class river quality system ranging from reference status to bad status. A pre-classification procedure was applied (Moog & Sharma 2005a), comprising (1) assessment of sensory features, (2) assessment of the abundance of bacteria, fungi and periphyton, and (3) screening of the benthic macroinvertebrate assemblage (Table 14). At least 17 sites per stream type and country were selected. A set ideally comprised four sampling sites pre-classified "high", four sites classified "good", three sites classified "moderate", three sites classified "poor" and three sites classified "bad". A detailed description of pre-classified sampling sites and its characteristics is provided for India (Appendix 2_1). Overall, 198 sampling sites were selected (Table 13).

Sampling

Sampling teams were trained in a standardized sampling procedure (based on Barbour *et al.* 1999 and AQEM consortium 2002) to guarantee for harmonized sampling effort in all countries. Sites were ideally sampled twice, post-monsoon (late October 2005 to December 2005) and pre-monsoon (mid March 2006 to early June 2006) resulting in 373 samples. Multi-habitat samples, reflecting the proportion of the microhabitat types present with ≥ 5% cover, were taken from each stream site. At each site 20 sample units were taken, each notionally of 25 cm x 25 cm dimensions, resulting in ca. 1.25 m² of stream bottom being sampled. Macroinvertebrates were sorted and identified to the lowest attainable taxonomic level (usually genus). Samples containing less than 20 specimens due to heavy pollution were not considered in the analysis. Consequently, the developed multimetric index can only be applied to rivers with a certain degree of “higher” life.

A total of 38 environmental parameters reflecting river perturbation were recorded at each sampling site (Table 15), either in the field, or based on the analysis of water samples, or using Geo Information System (GIS). The parameters were selected to reflect a range of different stressors from the four stressor types organic pollution, eutrophication, land-use in the catchment and degradation of hydromorphology. In addition a Land use Index and a Hydromorphology Index are calculated according to Hering *et al.* (2006b) and included into the analysis, with the Land use Index being calculated as “% urban areas + 0.5 * % cropland”, assuming that urban area more strongly influence the quality of streams than cropland, while the impact of forest and pasture is minimal; the Hydromorphology Index is calculated as “% shoreline covered with woody riparian vegetation + % no bank fixation + % no bed fixation - (stagnation * 100) - (straightening * 100)” reflecting habitat degradation.

Table 14: Parameters applied to pre-classify the impact of pollution according to Moog & Sharma (2005a). Simplified.

Assessment features	Parameter
Sensory features	Non natural turbidity Non natural colour Foam Odor Waste dumping
Ferro-sulphide reduction (lentic zones)	% mud with aerobic surface % mud with anaerobic surface % lower surface of stones % upper and lower surface of stones
Ferro-sulphide reduction (lotic zones)	% mud with aerobic surface % mud with anaerobic surface % lower surface of stones % upper and lower surface of stones
Bacteria, fungi, periphyton	Abundance of sewage fungi & bacteria (visible with the naked eye) Abundance of sulphur bacteria (visible with the naked eye) Abundance of stones with algal vegetation (periphyton) in thin layers % of thick, significant layers of algae Abundance of filamentous green algae
Benthic macroinvertebrates	Species richness (five abundance classes) Abundance of different clean water taxa according to BMWP/NEPBIOS (Sharma & Moog 2005) Abundance of different tolerant taxa (leeches, red Chironomids, Tubificidae, Air-breathing insects, Hydropsychidae)

Table 15: Environmental parameters used for PCA.

Stressor type	Abbrev.	Parameter	Transform.
Land-use in the catchment	D_FOR	% deciduous native forest	Arcsin sq.root
	C_FOR	% coniferous native forest	Arcsin sq.root
	FOR	% forest (D_FOR+M_FOR)	Arcsin sq.root
	N__UNV	% naturally unvegetated	Arcsin sq.root
	CROP	% crop land (tillage, lowland)	Arcsin sq.root
	PAST	% pasture	Arcsin sq.root
	O_GRA	% open grass-/bushland (natural)	Arcsin sq.root
	URB	% urban sites	Arcsin sq.root
	VILL	% villages	Arcsin sq.root
	LUI	Land use Index	Arcsin sq.root
	S_ZEN	% shading at zenith	Arcsin sq.root
	R_BED	Removal mineral bed material (yes/no)	Arcsin sq.root
Hydromorphology	W_RIP	% average density of wooded riparian vegetation	Arcsin sq.root
	BA_FIX	% bank fixation	Arcsin sq.root
	BE_FIX	% bed fixation	Arcsin sq.root
	R_VEG	Removal/lack of natural catchment vegetation (yes/no)	Log 10
	F_TYP	Number flow types	Log 10
	L_IMP	Longitudinal impoundments at sampling site (yes/no)	Log 10
	HYI	Hydromorphology Index	Arcsin sq.root
Organic pollution/eutrophica-tion	NS_POL	Non-source pollution (yes/no)	Log 10
	S_OVE	Sewage overflows (yes/no)	Log 10
	EUT	Signs of eutrophication (yes/no)	Log 10
	W_USE	Number of water uses	Log 10
	FISH	Fisheries (yes/no)	Log 10
	C_WAT	Cattle watering place (yes/no)	Log 10
	RUB	Rubbish (yes/no)	Log 10
	FAE	Faeces (yes/no)	Log 10
	WAS	Washing/bath (yes/no)	Log 10
	FOA	Foam (yes/no)	Log 10
	TUR	Turbidity (yes/no)	Log 10
	CON	Conductivity (µS/cm), field	Log 10
	OXY	Oxygen saturation (%), field	Log 10
	CHL	Chloride (mg/l), field, laboratory	Log 10
	BOD	BOD (mg/l), laboratory	Log 10
	NIT	Nitrate (mg/l), field, laboratory	Log 10
	O_PHO	Ortho-phosphate (µg/l), laboratory	Log 10
	E_COL	E-coli (n/100 ml), laboratory	Log 10
	AMM	Ammonium (mg/l), laboratory	Log 10

2.2.2 Gradient analysis

Principal Component Analysis (PCA) was applied to reduce the dimensionality of the environmental dataset and to define proxies for the impact of the addressed stressors. Parameters were transformed as shown in Table 15. As I aimed at the development of a stressor-specific assessment system, four individual PCA gradients for mountain and lowland streams were constructed, restricted to parameters reflecting the stressor types organic pollution, eutrophication, land-use in the catchment and degradation of hydromorphology. As the development of a stressor type specific analysis demands for sampling sites not severely impacted by a second stressor, threshold values were applied to exclude sampling sites heavily impacted by another stressor (BOD_5 > 6mg/l; Ortho-phosphate ≥ 700 µg/l for lowland and ≥ 600 µg/l for mountainous rivers; Land use Index > 40 for lowland and > 30 for mountainous rivers; Hydromorphology Index > 0). From each analysis I selected a) the two most important independent environmental parameters, i.e. strongest explanatory power in the PCA and b) the values of PCA axes 1 and 2 as independent variables for the selection of biotic indices. PCA analysis was conducted with 4.1 CanoDraw for Windows (1999-2003, Petr Smilauer), 4.51 CANOCO for Windows (1997-2003, F. ter Braak and P. Smilauer).

2.2.3 Metrics: Calculation and selection

Metric calculations

A total of 28 metrics (Table 16) were calculated with the taxalists of the 373 benthic invertebrate samples to be correlated against the selected environmental parameters. These metrics have been used in previous studies from other regions to detect river perturbation caused by organic pollution, eutrophication, hydromorphological impact and land-use of the catchment (Barbour *et al.* 1996, Sponseller *et al.* 2001, Lorenz *et al.* 2004, Sandin & Hering 2004). Metrics were based on relative abundance (%), abundance classes (Ab_cl) (Table 3) and individual numbers (No) and and were beforehand assigned to metric types: (1) Composition/abundance metrics (C/A-metrics) provide different classes of information. They may give the relative proportion of a taxon or taxonomic group with respect to its total number or abundance, respectively. The rationale is that a stable community under reference conditions will be relatively consistence in its proportional representation, though measuring of individual abundances may vary in magnitude. Abundance of key taxa, e.g. Ephemeroptera, Plecoptera and Trichoptera (EPT) provide information about the condition of the targeted assemblage. (2) Richness/diversity metrics (R/D-metrics) reflect the diversity of the community. They measure the availability of habitat, food source, and other requirements to support survival and propagation of many species. Increasing metric values correlate with increasing health of the community. Diversity metrics are number of families, genera, or lower taxa within a certain taxonomical entity, including all diversity indices. (3) Sensitivity/tolerance metrics (S/T-metrics) give taxa known to respond sensitively or tolerantly to a stressor or a single aspect of the stressor, respectively. (4) Functional metrics (F-metrics) give ecological function of taxa, e.g. feeding types, substrate preferences, current preferences, life cycle parameters and others. They reflect habitat type and quality of the observed river stretch and can be compared with the belonging situation under reference condition (Barbour *et al*. 1999, Hering *et al.* 2006a) (Table 16). Metrics were calculated with MICROSOFT OFFICE EXCEL 2003 SP3 (1985-2003, Microsoft Corporation).

Table 16: Metrics investigated. EPT = Ephemeroptera, Plecoptera, Trichoptera. Metrics EPT, Ephemeroptera, Plecoptera, Trichoptera and Diptera were applied on species (SP), genus (GEN) and family (FAM) level. Composition/abundance metrics on relative abundance level and individual abundance level. For rationale of metrics see chapter "Metric calculation" and "Discussion". Table continues page 55.

Metric type	Metric	Definition	Expected response to increasing stress
Composition/abundance (C/A-metrics)	EPT	Metric measures relative abundance of Ephemeroptera, Plecoptera and Trichoptera	Decrease
	Ephemeroptera	Metric measures relative abundance of Ephemeroptera	Decrease
	Trichoptera	Metric measures relative abundance of Trichoptera	Decrease
	Plecoptera	Metric measures relative abundance of Plecoptera	Decrease
	Diptera	Metric measures abundance of Diptera	Increase
Richness/diversity (R/D-metrics)	No Taxa	Metric counts all taxonomical units	Decrease
	No Families	Metric counts all families	Decrease
	No Genus	Metric counts all genera	Decrease
	No Species	Metric counts all species	Decrease
	No Individuals	Metric counts all individuals	Decrease
	No EPT-taxa	Metric measures abundance of Ephemeroptera, Plecoptera and Trichoptera	Decrease

Metric type	Metric	Definition	Expected response to increasing stress
	No Ephemeroptera taxa	Metric measures abundance of Ephemeroptera	Decrease
	No Trichoptera taxa	Metric measures abundance of Trichoptera	Decrease
	No Plecoptera taxa	Metric measures abundance of Plecoptera	Decrease
	No Diptera taxa	Metric measures abundance of Diptera	Increase
	Shannon-Wiener Diversity	Metric explains the homogeneity of taxa distribution (Shannon and Weaver 1976)	Decrease
	Evenness Diversity	Metric gives the maximum diversity of a given SHAN (Meschkowski 1968)	Decrease
	Margalef Diversity	Relation between all taxa to total amount of individuals (Margalef 1969)	Decrease
	Maximum Diversity	Metric gives the degree to which all taxonomical units are equal distributed (Meschkowski 1968)	Decrease
Sensitivity/tolerance (S/T-metrics)	Oligochaeta	Metric measures abundance of Oligochaeta	Increase
	Chironomidae	Metric measures abundance of Chironomidae individuals	Increase
	Batidae-Simuliidae-Hydropsychidae-Chironomidae	Metric counts Baetidae (BA),Simuliidae (SI), Hydropsychidae (HY) and Chironomidae (CH)	Increase
	BMWP and ASPT (NEPBIOS)	Biological Monitoring Working Party. Average Score Per Taxon. Taxa were classified according to their sensitivity to organic pollution (Armitage et al. 1983); BMWP and ASPT were used which were adapted by Sharma and Moog (2005) to the rivers in Nepal (NEPBIOS)	Decrease
Functional (F-metrics)	Pelal preference	Metric counts the taxonomical units with mud preferences	Decrease
	No Lithobiont	Metric counts the taxonomical units only living on stones	Decrease
	No Lithophil	Metric counts the taxonomical units living preferably on stones but also on other substrates	Decrease
	No Lithal	Metric counts the taxonomical units with stone preferences (Lithobiont + Lithophil)	Decrease
	No Lotic	Metric counts the taxonomical units with preferences to high flow velocities	Decrease

Selection of candidate metrics

Candidate metrics were selected for each stream type and stressor type separately. Metrics responding monotonically to the increase or decrease of environmental stress were considered most suitable. Frequency scatter plots in combination with Spearman rank correlation (threshold $r > 0.5$) were applied to correlate metrics against environmental parameters. In addition sampling sites were divided into stressed (threshold value 33% percentile) and unstressed (threshold value 67% percentile) sites with respect to a given stressor. A metric was selected as candidate if the interquartiles (25%/75% percentile) of stressed and unstressed sampling sites were not overlapping (Vlek *et al.* 2004). The aim was to select three metrics per metric type and stressor. If less than three metrics met this criterion, I considered metrics for which interquartile ranges of the 30%/70% percentile were not overlapping. This statistical procedure was restricted to ecologically meaningful metrics, i.e. those consistent with ecological principles and biological knowledge. Frequency scatterplots, Spearman rank correlation, and Box and Whisker plots were performed with STATISTICA 6.1 (1984-2003, StatSoft Inc).

Selection and processing of core metrics

To select core metrics to be included into the multimetric index from the set of candidate metrics the following rules were applied: (1) Core metrics should cover different metric types (see above), and (2) metrics should not give redundant information. Inter-correlation tests between candidate metrics were carried out to detect redundant metrics (threshold value Spearman's $r > 0.75$). Of correlating metrics I kept those increasing or decreasing with stress intensity in a more monotonic way (graphical analysis of scatterplots) and separating stressed and unstressed sampling sites more clearly (graphical analysis of Box and Whisker plots). Furthermore, I considered the overall correlation to the selected metrics.

The range of the selected core metrics was restricted through “upper and lower anchor values”, with the upper anchor (95% percentile) corresponding to reference conditions and the lower anchor (5% percentile) to heavily impacted sites. The different numerical scales of metrics (e.g. %, abundance class, number of individuals) were normalized to unitless scores between 0 and 1. Finally, a metric was selected as a core metric if 75% of the normalized metric values in sites pre-classified as "high" and "good" obtained values $\geq$ 0.5 (“75%-rule”) and if the coefficients of variance (C.V.) was < 1. The “75%-rule” in combination with the C.V. should guarantee for robust and reproducible core metrics (Figure 12).

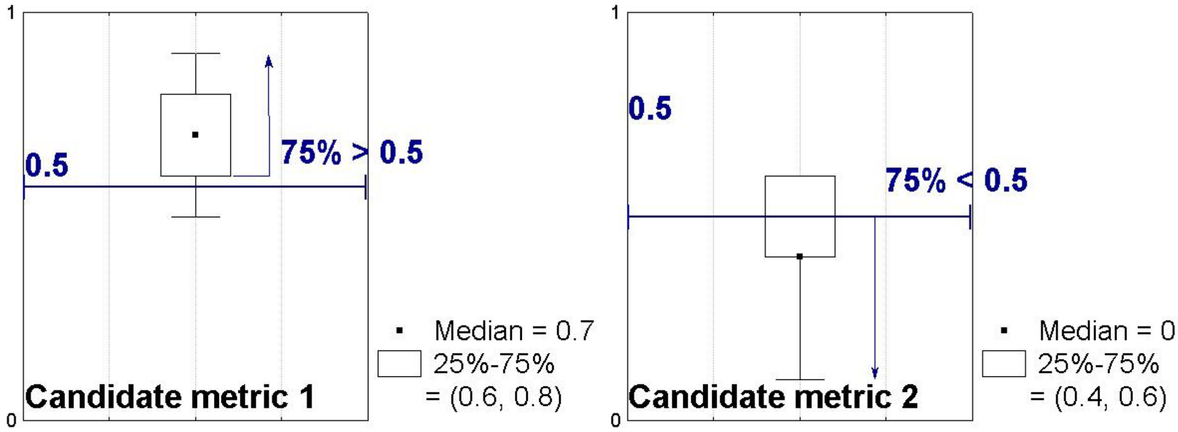


Figure 12: Example “75%-rule”. Candidate metric 1 passes threshold criteria, because 75% of its values are > 0.5. Candidate metric 2 failed, because more than 75% of its values are < 0.5 (25% percentile 0.4).

2.2.4 Calculation of the multimetric index

The multimetric index for the Hindu Kush-Himalaya region (“HKHindex”) was calculated as the mean of the normalized core metric results, which is the basis for a classification of sites into five quality classes (“high”, “good”, “moderate”, “poor”, and “bad”), expressing the deviation from reference conditions. The 25% percentile value of a single core metric under reference conditions (sites pre-classified “high”) was defined as threshold value for class "high". This value takes the natural variability of core metric values under reference conditions into account. The quality classes “good” to “bad” were evenly spread over the range that was left after setting the boundary for class “high”.

2.2.5 Seasonal effects

I tested if the core metrics are affected by seasonality or if metrics are robust enough to allow sampling during the entire dry season. Pre- and post-monsoon metric values were tested for differences (Mann-Whitney U-test). Only sampling sites that were pre-classified “high” or “good” (reference sites) were considered for this analysis.

Figure 13 summarizes the procedure for the development of the multimetric index.

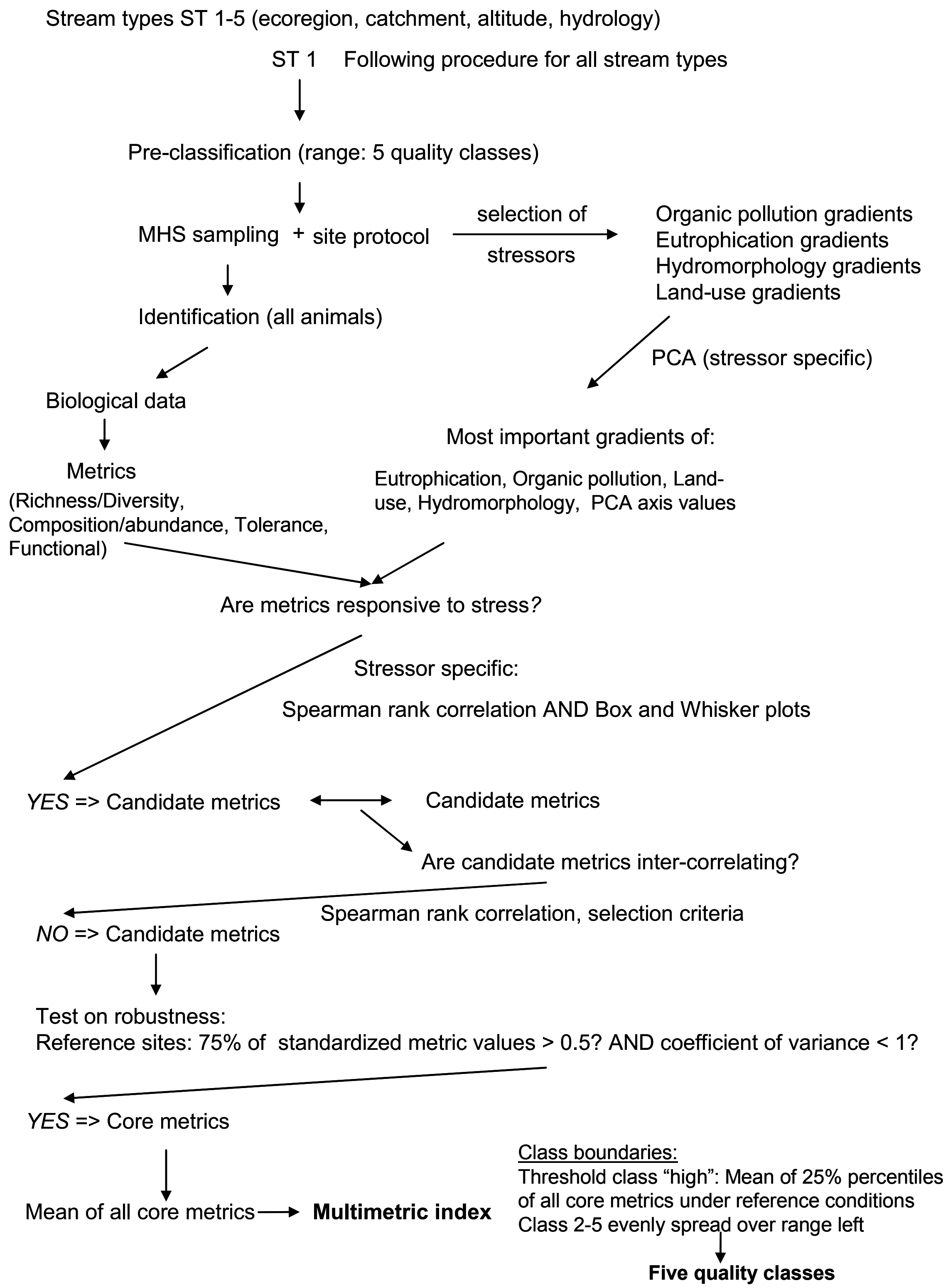


Figure 13: Procedure for the development of the multimetric index. MHS = Multi-Habitat sampling. PCA = Principal Component Analysis. Simplified.

2.3 Results

2.3.1 Gradient analysis

According to PCA results, the parameters that best reflect the variability in the environmental datasets are widely similar for mountain and lowland streams (Figure 14). BOD and E-coli are best reflecting the variability of the environmental parameters representing organic pollution. The vectors of these variables display nearly a right angle those for the mountain and for the lowland stream dataset, suggesting independence from each other and explaining different portions of the dataset's variability. In the lowland dataset E-coli and BOD are almost parallel to PCA axes 1 and 2, respectively, hence well reflecting the overall variability within the data set. In the mountain dataset the arrangements of sites parallel to the second PCA axis reveals another important gradient independent of the selected environmental parameters (Figure 14). Ortho-phosphate is best represented eutrophication stress, both in the lowland and mountain sampling sites, supplemented by nitrate (lowland) and conductivity (mountains) (Figure 14). The Landuse Index is generally best reflecting variability in the catchment land-use dataset, while % forest is the second important gradient for the mountain sampling sites and % cropland and % villages for the lowlands. Of the two latter, % villages was selected, because sampling sites are more lined up along this gradient than along % cropland (Figure 14). Average density of wooded riparian vegetation and % bank fixation are the most important gradients in the hydromorphological stressor group. These two parameters best explain the variability within both in the mountainous rivers and in the lowland rivers data set. (Figure 14). In addition to these parameters I used the PCA values of axis 1 and 2 as independent variables for subsequent analysis.

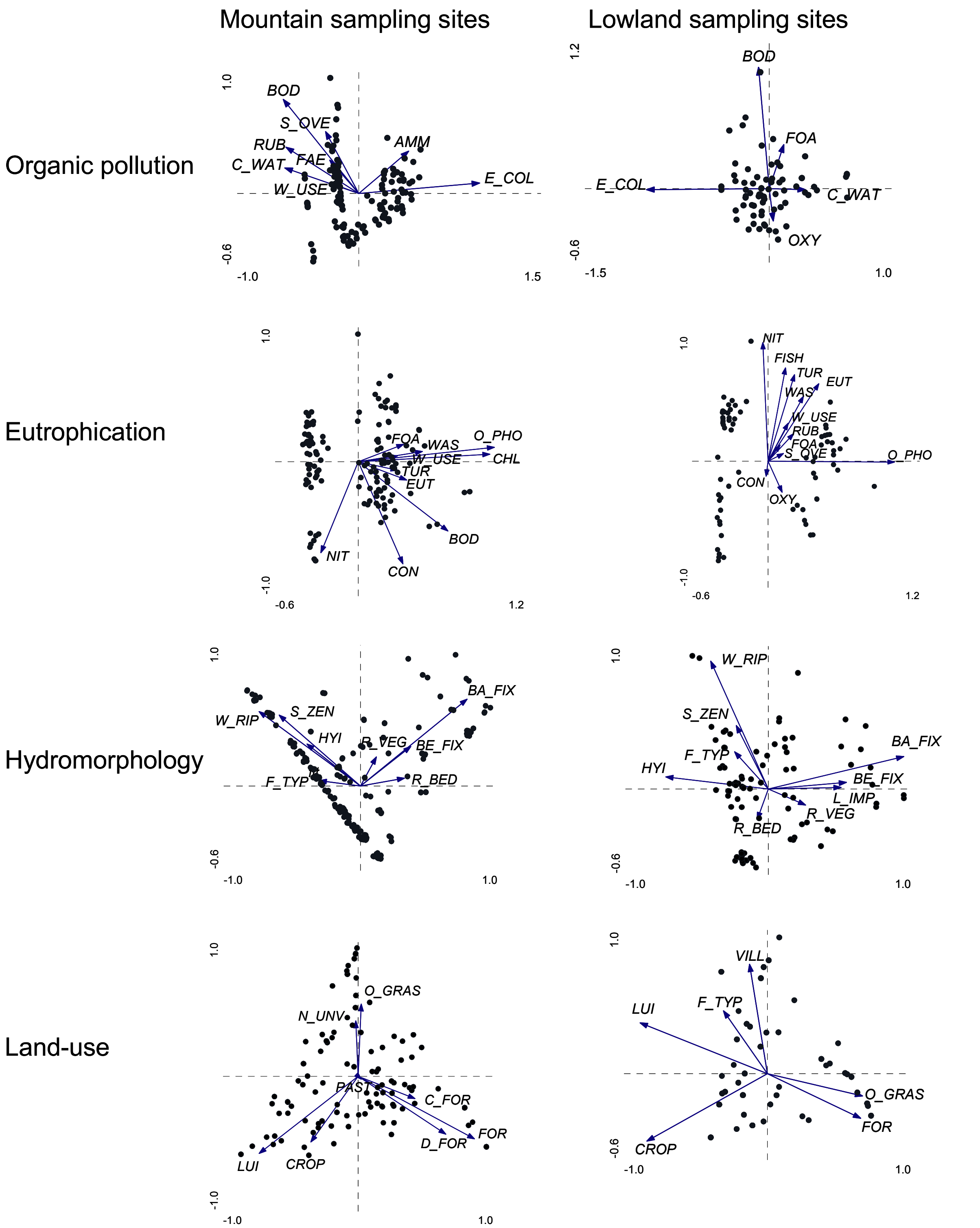


Figure 14: PCA biplots of environmental parameters representing different stressor types for lowland and mountain rivers. Arrows = environmental parameters. Circles = sampling sites. For abbreviations of environmental gradients see Table 15.

2.3.2 Metrics: Selection of candidate and core metrics

For each stream type at least six candidate metrics passed the threshold criteria (for figures of all candidate metrics see Appendix 2_2). However, only rivers of the Eastern Himalayan Broadleaf Forest reveal at least three metrics per stressor type. In all stream types the abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa decrease with the intensity of all four stressor types. In four out of five stream types (exception Lower Gangetic Plains) diversity indices qualify as candidate metrics for the detection of all stressor types. BMWP and ASPT were selected in all ecoregions as indicators to detect organic pollution and/or eutrophication. Only in the ecoregions of Eastern Himalayan Broadleaf Forest and Upper Gangetic Plains do the functional metrics respond to stream deterioration (Table 17).

Himalayan Subtropical Pine Forest

13 candidate metrics were detected which passed the threshold criteria. Nine metrics indicate eutrophication, eight organic pollution, and only one land-use of the catchment. % EPT FAM, No EPT FAM, Margalef Diversity, ASPT and BMWP detect both eutrophication and organic pollution. Altogether, eight candidate metrics are related to some aspect EPT. The three selected diversity indices mostly detect eutrophication and organic pollution (Table 17). Of the metrics addressing EPT, which were usually correlated, I selected % EPT FAM as core metric, because it reflects river perturbation caused by eutrophication and organic pollution (Table 17, Table 18, Figure 15). Of the correlated diversity indices I selected Margalef Diversity as it is slightly better correlating to organic pollution (Table 17, Table 18, Figure 15). Overall, seven metrics (two C/A-metrics, three R/D-metrics and two S/T-metric) were selected as potential core metrics, which mainly integrate the impact of organic pollution and eutrophication; in addition, only the metric “Ab_cl Plecoptera IND” reflects land-use intensity (Table 17).

The standardized values of three potential core metrics (% Ephemeroptera FAM, Ab_cl Plecoptera IND and Ab_cl Chironomidae IND) vary strongly between reference sites, i.e. less than 75% of standardized metric values increased 0.5 (“75%-rule”). Thus, they were removed from the set of candidate metrics leading finally to four core metrics (Table 23).

Eastern Himalayan Broadleaf Forest

Compared to the other mountainous ecoregions, the highest number of metrics responds significantly to river deterioration within this ecoregion resulting in 16 candidate metrics. For each stressor type at least four metrics were detected which are responsive to river perturbation. Again, most candidate metrics are derived from EPT (six metrics) detecting all four stressor types. The four diversity indices indicate eutrophication, hydromorphological impact and land-use of the catchment. Nine metrics detect more than one stressor type (Table 17). Seven metrics (three R/D-metrics, three S/T-metrics and one F-metric) were selected as potential core metrics, all of which are capable to indicate the impact all of the stressor types investigated (Table 17). As in other ecoregions, metrics addressing EPT taxa were highly inter-correlating (Table 19); I selected No EPT FAM (Figure 16) and No Ephemeroptera FAM as they best detect organic pollution, eutrophication, land-use pressure and hydromorphological degradation (Table 17). Of the highly correlated diversity indices (Table 19) I selected Shannon-Wiener Index, as it responds slightly stronger than other diversity indices (Table 17, Figure 16). Finally six core metrics are used for calculating of the multimetric index (Table 23).

Western Himalayan Broadleaf Forest

Only eight candidate metrics were obtained that respond to perturbation (Table 17). Inter-correlation tests revealed strong linkage between three tolerance metrics. (Table 20); the metric Ab_cl Baetidae-Simuliidae-Hydropsychidae-Chironomidae IND was selected as potential core metric as it reacts most strongly to eutrophication (Table 17, Figure 17). The core metrics detect eutrophication (six metrics), hydromorphological degradation (% Plecoptera FAM) and land-use pressure (Margalef Diversity) (Figure 17), and belong to three different metric types; two C/A-metrics, one R/D-metric and two S/T-metrics (Table 17, Table 23).

Upper Gangetic Plains

Altogether 16 candidate were obtained from the gradient-metric analysis, which mostly detect land-use in the catchment (14 metrics), belonging to all of the four metric types (Table 17). Nevertheless, out of the 16 candidate metrics only four metrics remained which are not inter-correlated with each other (Table 21). % Ephemeroptera FAM was selected from the set of EPT metrics, as it rarely correlates with other metrics (Table 21). Shannon-Wiener diversity was selected as diversity metric as it best separate stressed from unstressed sampling sites (Figure 18). Sampling sites which are heavily affected by land-use are clearly separated from unstressed sampling sites by BMWP (Figure 18). Consequently, the other sensitivity metrics, which are correlated with BMWP, were removed. The functional metric Ab_cl Lithobiont IND, which indicates land-use in the catchment, was removed from the analysis, because standardized metric values are highly variable in reference site leading finally to three core metrics (Table 23).

Lower Gangetic Plains

Only six candidate metrics were extracted from the gradient-metric analysis (Table 17). % EPT FAM (Figure 19) was kept as a potential core metric, because separation between stressed and unstressed sites is better than for No EPT GEN, especially if non-outlier range is also considered (Appendix 2_2). Finally three metrics remained as potential core metrics (one C/A-metric and two S/T-metrics). However, Ab_cl Oligochaeta is the only metric passing threshold criteria showing robust value, in contrast % EPT FAM and BMWP exhibit a large range under reference conditions (Table 23).

2.3.3 Calculation of the multimetric Index

Table 24 gives the range of the indices for each of the five river quality classes. Natural variability between stream types for class boundary between “high” and “good” ranges from 0.5 to 0.68.

2.3.4 Seasonal effects

Of 22 core metrics only two show significant abundance differences in reference sites between pre-monsoon and post-monsoon season (Mann-Whitney U-Test), namely % EPT IND and Ab_cl Baetidae-Simuliidae-Hydropsychidae-Chironomidae in the Western Himalayan Broadleaf Forest (Table 23).

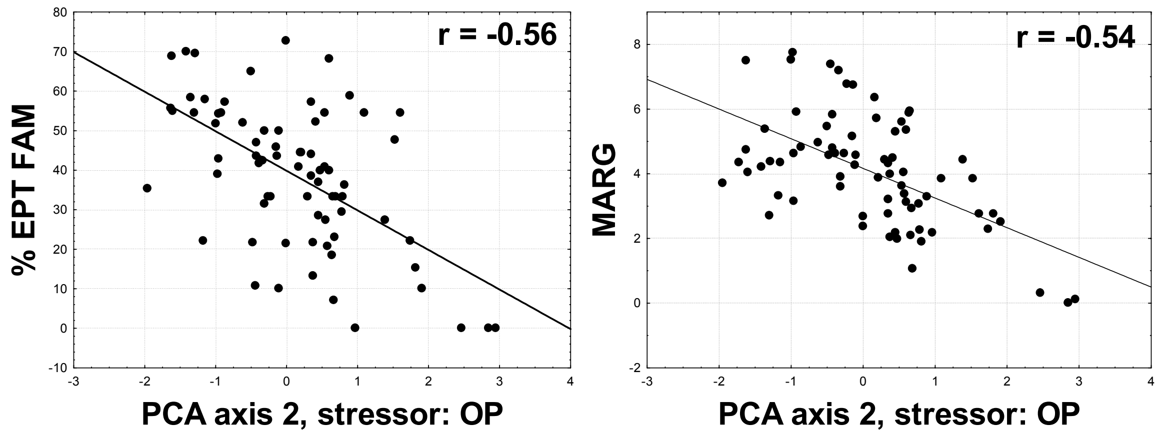


Figure 15: Example core metrics Himalayan Subtropical Pine Forest. OP = Organic pollution. MARG = Margalef Diversity. r = Spearman rank correlation.

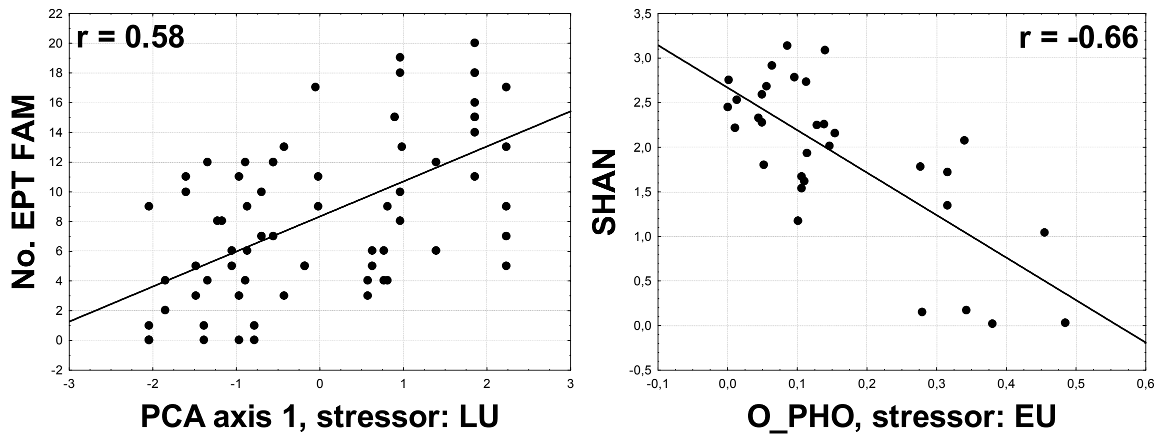


Figure 16: Example core metrics Eastern Himalayan Broadleaf Forest. LU = Land-use. EU = Eutrophication. SHAN = Shannon-Wiener Diversity. O_PHO = Ortho phosphate. r = Spearman rank correlation.

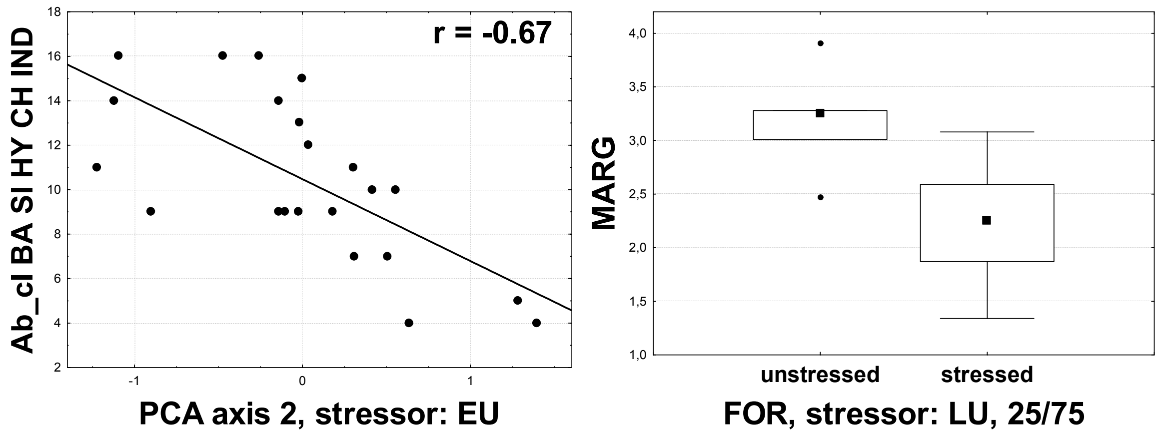


Figure 17: Example core metrics Western Himalayan Broadleaf Forest. Ab_cl = Abundance class. EU = Eutrophication. FOR = % forest catchment. LU = Land-use. BA SI HY CH = Baetidae-Simuliidae-Hydropsychidae-Chironomidae. MARG = Margalef Diversity. 25/75 = Box shows 25%-75% Interquartile range. r = Spearman rank correlation.

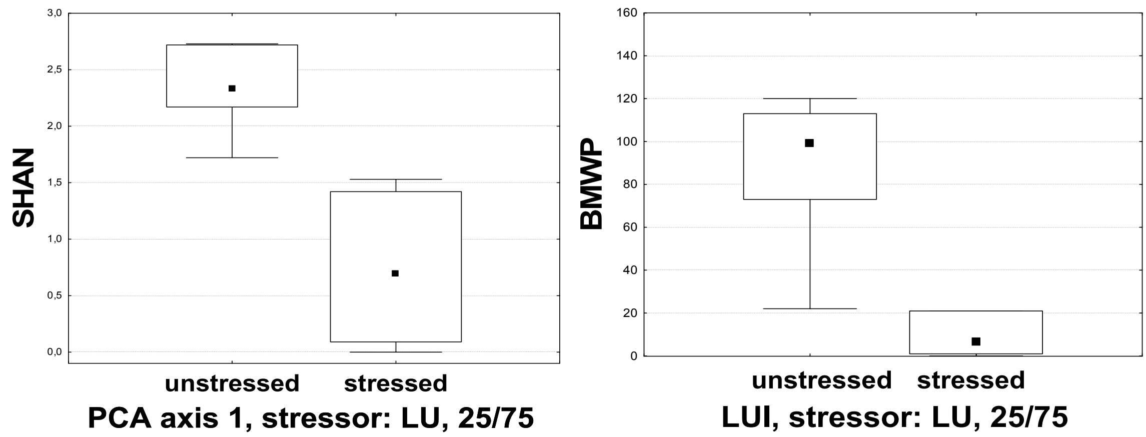


Figure 18: Example core metrics Upper Gangetic Plains. LU = Land-use. LUI = Landuse Index. SHAN = Shannon-Wiener Diversity. 25/75 = Box shows 25%-75% Interquartile range.

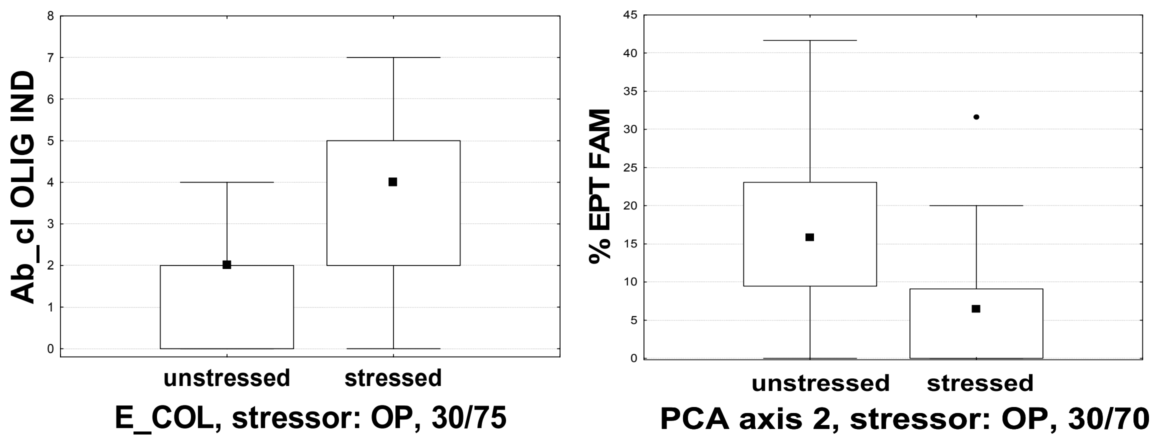


Figure 19: Example core metrics Lower Gangetic Plains. Ab_cl = Abundance class. E_COL = E-coli counts. OP = Organic pollution. OLIG = Oligochaeta. 30/70 = Box shows 30%-70% Interquartile range.

Table 17: Candidate metrics per stream type and stressor type. x = metrics meeting the “non-overlapping (n-o)” criteria with at least one environmental gradient of the according stressor type. Corresponding figures in the Appendix 2_2. Ab_cl = Abundance class. Table continues page 67-68.

Candidate metrics	Metric type	Spearman r > 0.5 and continuous increase or decrease				Non overlapping of 25%/75% interquartile				Non overlapping of 30%/70% interquartile			
		Eutro-phication (EU)	Organic pollution (OP)	Hydromor-phology (H-M)	Land-use (LU)	EU	OP	H-M	LU	EU	OP	H-M	LU
Himalayan Subtropical Pine Forest													
% EPT FAM	C/A	-0.56	-0.56										
% Trichoptera FAM	C/A	-0.57											
% Ephemeroptera FAM	C/A										x		
No EPT FAM	R/D		-0.52			x							
No Ephemeroptera GEN	R/D						x						
No Trichoptera FAM	R/D					x							
Margalef Diversity	R/D		-0.54			x							
Shannon-Wiener Diversity	R/D		-0.51										
Evenness	R/D					x							
Ab_cl Plecoptera IND	R/D								x				
Ab_cl Chironomidae IND	S/T					x							
ASPT	S/T					x	x						
BMWP	S/T					x					x		
Eastern Himalayan Broadleaf Forest													
% Trichoptera IND	C/A					x							
No EPT FAM	R/D			-0.66	0.58								
No Ephemeroptera FAM	R/D	-0.57					x	x					
No Trichoptera FAM	R/D							x					
No Trichoptera GEN	R/D							x					
No Families	R/D				0.62	x							
Evenness	R/D					x		x	x				

Candidate metrics	Metric type	Spearman r > 0.5 and continuous increase or decrease				Non overlapping of 25%/75% interquartile				Non overlapping of 30%/70% interquartile			
		Eutro-phication (EU)	Organic pollution (OP)	Hydromor-phology (H-M)	Land-use (LU)	EU	OP	H-M	LU	EU	OP	H-M	LU
Margalef Diversity	R/D	-0.63		-0.65	0.69								
Maximum Diversity	R/D							x					
Shannon-Wiener Diversity	R/D	-0.66			0.65			x					
Ab_cl Diptera IND	R/D					x							
Ab_cl Plecoptera IND	R/D			-0.66		x	x						
Ab_cl Oligochaeta IND	S/T	0.62											
Ab_cl Chironomidae IND	S/T					x							
ASPT	S/T	-0.65					x						
Ab_cl Lithophil IND	F	-0.62					x	x	x				
Western Himalayan Broadleaf Forest													
% Plecoptera FAM	C/A							x					
% EPT IND	C/A					x							
% Diptera IND	C/A					x							
Ab_cl Diptera IND	R/D	-0.62											
Margalef Diversity	R/D								x				
Ab_cl Batidae-Simuliidae-Hydropsychidae-Chironomidae IND	S/T	-0.67											
Ab_cl Chironomidae IND	S/T	-0.66											
ASPT	S/T					x							
Upper Gangetic Plains													
% EPT FAM	C/A								x				
% Trichoptera FAM	C/A								x				
% Ephemeroptera FAM	C/A								x				
% Diptera FAM	C/A												x
Ab_cl Diptera IND	R/D					x		x					
Ab_cl Ephemeroptera IND	R/D								x				

Candidate metrics	Metric type	Spearman r > 0.5 and continuous increase or decrease				Non overlapping of 25%/75% interquartile				Non overlapping of 30%/70% interquartile			
		Eutro-phication (EU)	Organic pollution (OP)	Hydromor-phology (H-M)	Land-use (LU)	EU	OP	H-M	LU	EU	OP	H-M	LU
No EPT FAM	R/D								x				
No Trichoptera FAM	R/D								x				
Evenness	R/D								x				
Shannon-Wiener Diversity	R/D								x				
BMWP	S/T								x				
ASPT	S/T								x				
Ab_cl Chironomidae IND	S/T							x					
Ab_cl Batidae-Simuliidae-Hydropsychidae-Chironomidae IND	S/T								x				
Ab_cl Lithal IND	F								x				
Ab_cl Lithobiont IND	F								x				
Lower Gangetic Plain													
% EPT FAM	C/A										x		
Ab_cl Ephemeroptera IND	R/D						x						
No EPT GEN	R/D					x	x						
No Families	R/D												x
Ab_cl Oligochaeta IND	S/T										x		
BMWP	S/T												x

Table 18: Spearman rank correlation coefficients (r) of candidate metrics for the ecoregion Himalayan Subtropical Pine Forest. r > 0.75 with asterisks. EPT = Ephemeroptera, Plecoptera, Trichoptera, TRICH = Trichoptera, EPH = Ephemeroptera, PLEC = Plecoptera, MARG = Margalef Diversity, SHAN = Shannon-Wiener Diversity, EVEN = Evenness, CHIRO = Chironomidae. Ab_cl = Abundance class.

Himalayan Sub-tropical Pine Forest	% EPT FAM	% TRICH FAM	% EPH FAM	Ab_cl PLEC	No EPT GEN	No EPT FAM	No EPH GEN	No TRICH FAM	MARG	SHAN	EVEN	ASPT	Ab_cl CHIRO	BMWP
% EPT FAM	1.00													
% TRICH FAM	0.85*	1.00												
% EPH FAM	0.66	0.30	1.00											
Ab_cl PLEC	0.45	0.43	0.03	1.00										
No EPT GEN	0.56	0.64	0.33	0.36	1.00									
No EPT FAM	0.78*	0.85*	0.42	0.53	0.78*	1.00								
No EPH GEN	0.65	0.63	0.54	0.47	0.74	0.87*	1.00							
No TRICH FAM	0.74	0.93*	0.23	0.49	0.75*	0.95*	0.74	1.00						
MARG	0.40	0.53	0.18	0.54	0.59	0.79*	0.80*	0.73	1.00					
SHAN	0.46	0.52	0.30	0.44	0.57	0.72	0.75*	0.66	0.87*	1.00				
EVEN	0.39	0.33	0.36	0.31	0.27	0.43	0.46	0.38	0.57	0.85*	1.00			
ASPT	0.70	0.65	0.31	0.57	0.52	0.64	0.52	0.63	0.38	0.39	0.31	1.00		
Ab_cl CHIRO	-0.26	-0.19	-0.10	-0.40	-0.07	-0.09	-0.09	-0.11	-0.11	-0.17	-0.37	-0.25	1.00	
BMWP	0.47	0.62	0.16	0.54	0.75	0.85	0.81	0.81	0.90	0.79	0.46	0.49	-0.08	1.00

Table 19: Spearman rank correlation coefficients (r) of candidate metrics for the ecoregion Eastern Himalayan Broadleaf Forest. r > 0.75 with asterisks TRICH = Trichoptera. EPT = Ephemeroptera, Plecoptera, Trichoptera, EPH = Ephemeroptera, PLEC = Plecoptera, EVEN = Evenness, HMAX = Maximum Diversity, MARG = Margalef Diversity, SHAN = Shannon-Wiener Diversity, OLIG = Oligochaeta, CHIRO = Chironomidae, LPHIL = Lithophil. Ab_cl = Abundance class.

Eastern Himalayan Broadleaf Forest	% TRICH IND	No EPT FAM	No EPH FAM	No PLEC FAM	No TRICH GEN	No FAM	Ab_cl DIP	Ab_cl PLEC	EVEN	HMAX	MARG	SHAN	Ab_cl OLIG	Ab_cl CHIRO	ASPT	BMWP	Ab_cl LPHIL
% TRICH IND	1.00																
No EPT FAM	0.76*	1.00															
No EPH FAM	0.46	0.69	1.00														
No PLEC FAM	0.18	0.79*	0.48	1.00													
No TRICH GEN	0.92*	0.91*	0.44	0.60	1.00												
No FAM	0.00	0.88*	0.80*	0.68	0.74	1.00											
Ab_cl DIP	-0.10	0.24	0.04	0.03	0.29	0.14	1.00										
Ab_cl PLEC IND	0.54	0.76*	0.53	0.95*	0.55	0.69	0.03	1.00									
EVEN	0.19	0.29	0.42	0.43	0.13	0.36	-0.27	0.50	1.00								
HMAX	0.56	0.91*	0.80*	0.71	0.78*	0.97*	0.23	0.72	0.35	1.00							
MARG	0.59	0.85*	0.79*	0.73	0.71	0.94*	0.01	0.74	0.51	0.95*	1.00						
SHAN	0.04	0.70	0.74	0.70	0.51	0.79*	-0.08	0.75*	0.82*	0.79*	0.88*	1.00					
Ab_cl OLIG IND	-0.38	-0.26	-0.12	-0.29	-0.23	-0.08	0.27	-0.31	-0.20	-0.11	-0.24	-0.20	1.00				
Ab_cl CHIRO IND	-0.16	0.08	-0.09	-0.09	0.14	-0.01	0.82*	-0.11	-0.38	0.06	-0.14	-0.23	0.36	1.00			
ASPT	0.03	0.75*	0.28	0.70	0.70	0.48	0.28	0.66	0.17	0.56	0.52	0.43	-0.51	0.10	1.00		
BMWP	0.01	0.95*	0.73	0.82*	0.82*	0.94*	0.11	0.80*	0.38	0.94*	0.93*	0.78*	-0.25	-0.07	0.67	1.00	
Ab_cl LPHIL	0.15	0.68	0.74	0.59	0.48	0.76*	-0.07	0.60	0.40	0.78*	0.79*	0.72	-0.15	-0.16	0.33	0.73	1.00

Table 20: Spearman rank correlation coefficients (r) of candidate metrics for the ecoregion Western Himalayan Broadleaf Forest. r > 0.75 with asterisks. PLEC = Plecoptera, EPT = Ephemeroptera, Plecoptera, Trichoptera, DIP = Diptera, MARG = Margalef Diversity, BA-SI-HY-CH =Baetidae-Simuliidae-Hydropsychidae-Chironomidae, CHIRO = Chironomidae. Ab_cl = Abundance class.

Western Himalayan Broadleaf Forest	% PLEC FAM	% EPT IND	% DIP IND	Ab_cl DIP	MARG	ASPT	Ab_cl BA SI HY CH	Ab_cl CHIRO
% PLEC FAM	1.00							
% EPT IND	0.05	1.00						
% DIP IND	-0.08	-0.99*	1.00					
Ab_cl DIP	-0.20	-0.59	0.63	1.00				
MARG	0.20	0.03	-0.07	0.00	1.00			
ASPT	0.26	0.26	-0.28	-0.07	0.01	1.00		
Ab_cl BA-SI-HY-CH	-0.24	-0.56	0.60	0.88*	0.00	-0.23	1.00	
Ab_cl CHIRO	-0.30	-0.71	0.76*	0.80*	-0.15	-0.33	0.78*	1.00

Table 21: Spearman rank correlation coefficients (r) of candidate metrics for the ecoregion Upper Gangetic Plains. r > 0.75 with asterisks EPH = Ephemeroptera, EPT = Ephemeroptera, Plecoptera, Trichoptera, TRICH = Trichoptera, SHAN = Shannon-Wiener Diversity, EVEN = Evenness, LBIO = Lithobiont, LITH = Lithal. Ab_cl = Abundance class.

Upper Gangetic Plains	% EPH FAM	% EPT FAM	% TRICH FAM	No TRICH FAM	Ab_cl EPH IND	No EPH FAM	No EPT FAM	SHAN	EVEN	ASPT	BMWP	Ab_cl LBIO	Ab_cl LITH
% EPH FAM	1.00												
% EPT FAM	0.82*	1.00											
% TRICH FAM	0.56	0.91*	1.00										
No TRICH FAM	0.53	0.86*	0.93*	1.00									
Ab_cl EPH IND	0.72	0.76*	0.67	0.77*	1.00								
No EPH FAM	0.77*	0.78*	0.64	0.75	0.92*	1.00							
No EPT FAM	0.72	0.86*	0.78*	0.90*	0.91*	0.92*	1.00						
SHAN	0.57	0.76*	0.75*	0.76*	0.62	0.61	0.74	1.00					
EVEN	0.32	0.46	0.45	0.36	0.22	0.20	0.30	0.79*	1.00				
ASPT	0.71	0.90*	0.91*	0.80*	0.55	0.50	0.66	0.80*	0.54	1.00			
BMWP	0.62	0.65	0.62	0.80*	0.89*	0.85*	0.88*	0.63	0.27	0.56	1.00		
Ab_cl LBIO	0.55	0.73	0.72	0.77*	0.75*	0.70	0.78*	0.69	0.32	0.66	0.68	1.00	
Ab_cl LITH	0.68	0.81*	0.73	0.80*	0.82*	0.78*	0.84*	0.76*	0.40	0.73	0.76*	0.90*	1.00

Table 22: Spearman rank correlation coefficients (r) of candidate metrics for the ecoregion Lower Gangetic Plains. r > 0.75 with asterisks, EPT = Ephemeroptera, Plecoptera, Trichoptera, EPH = Ephemeroptera. OLIG = Oligochaeta. Ab_cl = Abundance class.

Lower Gangetic Plains	% EPT FAM	No EPT GEN	No FAM	No EPH IND	BMWP	Ab_cl OLIG
% EPT FAM	1.00					
No EPT GEN	0.91*	1.00				
No FAM	0.12	0.43	1.00			
No EPH IND	0.78*	0.85*	0.42	1.00		
BMWP	0.20	0.46	0.89*	0.52	1.00	
Ab_cl OLIG	-0.28	-0.12	0.36	-0.15	0.23	1.00

Table 23: Statistics of potential core metrics; final core metrics in italics. UA = upper anchor value (reference), LA = lower anchor value (heavily stressed). EU = eutrophication, OP = organic pollution, H-M = hydromorphological degradation, LU = land-use of the catchment; p-level derived from comparison of metric values between pre- and post monsoon. W_RIP = % average density wooded riparian vegetation. BA-SI-HY-CH = Baetidae-Simuliidae-Hydropsychidae-Chironomidae. Ab_cl = Abundance class.

Metrics / Stream type	Stressor	"75%-rule"	C.V.	UA	LA	p-level
Himalayan Subtropical Pine Forest						
% EPT FAM	PCA axis 2, OP	0.61	0.21	68.8	0	0.09
	Conductivity	0.57				
% Ephemeroptera FAM	E-coli	0.49				
Ab_cl Plecoptera IND	PCA axis 1, LU	0.25				
Margalef Diversity	PCA axis 2, OP	0.52	0.42	7.4	1.07	0.48
	Conductivity	0.5				
Evenness	Conductivity	0.67	0.25	0.86	0.23	0.63
ASPT	Conductivity	0.55	0.16	7.5	1	0.54
	PCA axis 2, OP	0.59				
Ab_cl Chironomidae IND	Conductivity	0.43				
Eastern Himalayan Broadleaf Forest						
No EPT FAM	PCA axis 1, H-M	0.39				
	PCA axis 1, LU	0.34				
No Ephemeroptera FAM	Ortho-phosphate	0.67	0.3	6	0	0.71
Shannon-Wiener Diversity	Ortho-phosphate	0.73				0.29
	W_RIP	0.73				
ASPT	PCA axis 1, OP	0.86	0.04	7	1	0.34
	Ortho-phosphate	0.91				
Ab_cl Oligochaeta	Ortho-phosphate	0.57	0.39	0	7	0.34
Ab_cl Chironomidae	Ortho-phosphate	0.5	0.7	0	6	0.55
Ab_cl Lithophil IND	Land use Index	0.63	0.23	12	0	0.62
	W_RIP	0.58				
	Ortho-phosphate	0.5				
	PCA axis 2, OP	0.5				
Western Himalayan Broadleaf Forest						
% Plecoptera FAM	PCA axis 1, H-M	0.5	0.72	14.2	0	0.14
% EPT IND	PCA axis 2, EU	0.75	0.35	14.2	0	< 0.01
Margalef Diversity	% forest in the catchment	0.7	0.21	3.92	0.8	0.25
ASPT	PCA axis 2, EU	0.5	0.24	7.3	1	0.52
Ab_cl BA-SI-HY-CH	PCA axis 2, EU	0.5	0.73	4	16	< 0.01
Upper Gangetic Plains						
% Ephemeroptera FAM	PCA axis 1, LU	0.66	0.24	33.3	0	0.13
Shannon-Wiener Diversity	PCA axis 1, LU	0.69	0.32	2.73	0.9	0.67
BMWP	Land use Index	0.52	0.15	146	0	0.61
Ab_cl Lithobiont IND	W_RIP	0.25				
Lower Gangetic Plains						
% EPT FAM	PCA axis 2, OP	0.35				
BMWP	Land use Index	0.38				
Ab_cl Oligochaeta IND	E-coli	0.5	0.5	0	6	0.59

Table 24: Water quality class boundaries.

Ecoregion/Water quality class	"High"	"Good"	"Moderate"	"Poor"	"Bad"
Himalayan Subtropical Pine Forest	≥ 0.57	≥ 0.43	≥ 0.29	≥ 0.15	< 0.15
Western Himalayan Broadleaf Forest	≥ 0.59	≥ 0.44	≥ 0.29	≥ 0.14	< 0.14
Eastern Himalayan Broadleaf Forest	≥ 0.68	≥ 0.51	≥ 0.34	≥ 0.17	< 0.17
Upper Gangetic Plains	≥ 0.62	≥ 0.46	≥ 0.3	≥ 0.14	< 0.14
Lower Gangetic Plains	≥ 0.5	≥ 0.37	≥ 0.24	≥ 0.11	< 0.11

2.4 Discussion

2.4.1 Metrics: Selection of core metrics

The selection of EPT taxa as core metric in all stream types underlines the worldwide suitability of this group for integrative river quality assessment and is in consistence with studies from different regions, e.g. Barbour *et al.* 1999 (North-America), Maxted *et al.* 2000 (North America), Sandin & Hering 2004 (Europe), Ollis *et al.* 2006 (South-Africa), Baptista *et al.* 2007 (South-America). In four of the five stream types diversity indices were selected as core metrics. Since diversity indices depend on the quality and availability of habitats (Barbour *et al.* 1999) they reflect the impact of all investigated stressors independent of ecoregion boundaries. The metrics ASPT and BMWP were selected in all stream types as sensitivity metrics. Both metrics are most suitable to detect organic pollution also in other regions, e.g. in Europe (Sandin & Hering 2004). The metric Ab_cl Baetidae-Simuliidae-Hydropsychidae-Chironomidae was developed to detect organic pollution, with which the abundance of these families may increase (Buss *et al.* 2002, Buss & Salles 2007). Suspension feeders (Hydropsychidae and Simuliidae), grazers (Baetidae) and oxygen depletion resistant collectors (many Chironomidae) may benefit from eutrophication and organic pollution and may be summed up to reflect pollution status. Only two functional metrics describing habitat preferences have been identified as core metrics. Substrate and current preferences are the only functional metrics available for benthic macroinvertebrates in the Hindu Kush-Himalaya region (see part one of this thesis). Other potentially suited functional metrics such as feedings types (e.g. Rawer-

Jost *et al.* 2000, Kerans & Karr 1994) have yet not been defined for the Hindu Kush-Himalaya fauna.

2.4.2 Development of the multimetric index

Selection and pre-classification of sampling sites was partly based on the biota (Table 14); thus, the development of the multimetric index included a circular component. However, the selection of core metrics depended solely on correlation with environmental parameters.

Metric values under reference conditions are comparable to values obtained by Barbour *et al.* (1996), Maxted *et al.* (2000), Ofenböck *et al.* (2004) and Baptista *et al.* (2007). When defining class boundaries for reference conditions the natural range of a metric must be taken into account (e.g. Bailey *et al.* 2004). Generally, I followed the approach used in North America (e.g. Barbour *et al.* 1996) and Europe (Hering *et al.* 2006a) and used the 25% percentile values of metrics from reference sites as the reference class boundary. Additionally, core metric were restricted to metrics with 75% of its standardized values in reference sites $\geq$ 0.5 and C.V. < 1. The five different class boundaries for class "high" range between 0.5 and 0.68 exhibiting a relatively low threshold value for references. However, each definition to set the "reference" boundary for a metric is artificial, because variability of macroinvertebrate communities and its patterns are multidimensional, and could hence not be defined by a single value.

The development of a stressor type specific multimetric index was not possible for all stream types, as the pre-selection of sampling sites mainly accounted for pollution parameters and did not target a hydromorphological gradient specifically. Nevertheless, the resulting sampling sites exhibit land-use and hydromorphological gradients, which are reflected by several metrics: Number and proportion of EPT taxa (Plecoptera in particular), Shannon-Wiener and Margalef Diversity indices, BMWP, and number of lithobiont taxa. Hydromorphological degradation mainly reflects habitat availability (e.g. loss of wooded riparian vegetation or loss of bank habitats due to fixation); the biotic metrics mentioned above are all related to habitat availability, as a low number of habitats leads to less niches for invertebrate families. This also affects metrics such as BMWP which are primarily reflecting river pollution; however, several

EPT taxa being sensitive to pollution are affected by habitat availability, too. For the Lower Gangetic Plains only a single metric (number of Oligochaeta) was robust, most likely due to the lack of reference sites in the sampling scheme.

2.4.3 Seasonal effects

Brewin *et al.* (2000) investigated seasonal effects of the monsoon climate on the abundance of benthic macroinvertebrates mountain streams in Nepal; in pristine sampling sites abundance did not change from post-monsoon to pre-monsoon season. The results confirmed these findings, because all metrics in the mountainous rivers (500-2500 m) do not show significant differences between post- and pre-monsoon season. There are two exceptions: % EPT IND and Ab_cl Baetidae-Simuliidae-Hydropsychidae-Chironomidae show significant seasonal abundance differences in the Western Himalayan Broadleaf Forest (Table 23). However, for % EPT IND the interquartile range of pre- and post monsoon season broadly overlap. The increased abundances of Ab_cl Baetidae-Simuliidae-Hydropsychidae-Chironomidae in the post monsoon season may be caused by fast development rates of these taxa, leading to more than one generation within one dry season and to an abundance peak in the post-monsoon season.

For the application of the HKH index sampling is possible through the entire dry season. Nevertheless, I propose sampling in the pre-monsoon season at times of minimum discharge, maximum pollutant concentration and water temperatures.

3 Summary and Conclusion

This thesis investigates the ecology of benthic macroinvertebrates inhabiting rivers in the Hindu Kush-Himalaya region, and covered the countries Bangladesh, Bhutan, Nepal, India, and Pakistan. In these countries, river monitoring focuses on physical and chemical parameters, pollutants, and other human toxic substances. Only in India and Nepal biological monitoring is applied using the ASPT (Armitage *et al.* 1983) adapted to the indigenous rivers. In none of the countries exists a comprehensive assessment method to evaluate rivers as ecosystems, which are mainly ruled by various anthropogenic influences. In addition, the knowledge of benthic macroinvertebrates for biological monitoring purposes is still incomplete. On this perceived need, two objectives become central issues of this thesis: To investigate substrate and current preferences of benthic macroinvertebrates as background information for river assessment, and to develop a multimetric assessment system for ecological river evaluation in the Hindu Kush-Himalaya region.

In the post-monsoon season of 2005 and the pre-monsoon season of 2006 two standardized sampling procedures were carried out at 198 rivers in Bangladesh, Bhutan, Nepal, India, and Pakistan covering five different ecoregions (based on the WWF Global 2000 ecoregions, Olson *et al.* 2001). To obtain ecological information on benthic macroinvertebrates a substrate specific sampling was conducted in near-natural sampling sites resulting in 271 samples. Substrates were sampled with respect to their relative contribution to riverbed material focusing on the dominant substrate types. In addition, at each sampling site a protocol was applied to record substrate type, current velocity, depth, and distance from shoreline. For the development of the multimetric assessment system a Multi-Habitat sampling (based on Barbour *et al.* 1999 and AQEM consortium 2002) was conducted at 373 sampling sites covering a gradient from reference sites to heavily impacted sites (Moog & Sharma 2005a). Environmental parameters describing organic pollution, eutrophication, land-use, and hydromorphology were also recorded at each sampling site.

Substrate and Current Preferences of Benthic Macroinvertebrates as Impact Indicators of Hydromorphological Degradation

The main results of this investigation are summarized in the following:

- Substrate type and current velocity are the most important explanatory variables for the distribution of taxa in pristine lower mountain rivers and lowland rivers.
- 50 taxa of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Diptera, Odonata, Mollusca, and Oligochaeta were detected with significant substrate and/or current preferences. 34 taxa show preferences for stony substrate types mainly comprising Ephemeroptera, and Trichoptera taxa. 12 Mollusca taxa are assigned to muddy and sandy substrate types. 11 taxa show significant preferences for faster current velocities of which eight are Trichoptera taxa.
- Taxa showing significant substrate or current preferences are assigned to four different metrics. 34 significant stone dwelling taxa are assigned to the metric Lithal. The metric Lithobiont comprises 13 taxa which were exclusively found on stones. 21 taxa are allocated to the metric Lithophil, which exhibit significant preferences for stones but are also found on other substrate types. The metric Lotic consists of 11 taxa with preferences for faster current velocities.
- The metrics Lithal, Lithophil and Lotic are significantly correlated to the degree of hydromorphological impact. The metric Lithobiont has a weaker discriminatory power compared to the other metrics. Best results are obtained on abundance class level. Threshold values for the metrics were defined to differentiate between sampling sites which are stressed or unstressed by hydromorphological deterioration.
- A 20 point system was developed to quantify substrate and current preferences. Most taxa prefer small stones (6-20 cm) as life space. Seven taxa of Ephemeroptera and Trichoptera were detected showing low ecological potential to habitat alteration; hence they are suitable to serve as bioindicators. Most taxa prefer moderate current velocities (11-50 cm/s).

Development of a Multimetric Index for Ecological River Quality Assessment

The main results in terms of the development of an ecological river quality assessment method are summarized in the following:

- For five different stream types of the Hindu Kush-Himalaya region a multimetric assessment system was developed separately, each containing a set of robust core metrics. The number of core metrics which built the multimetric indices range between three and six core metrics per stream type.
- Each multimetric index is able to detect anthropogenic impacts on the river ecosystem caused by organic pollution, eutrophication, land-use in the catchment, and hydromorphological degradation.
- The range of the index under reference conditions was defined, accounting for the natural range of standardized metric values. A separate five class river quality system was generated for each stream type.
- In all stream types metrics comprising Ephemeroptera, Plecoptera and Trichoptera taxa are most suitable for river assessment. Diversity metrics and ASPT, BMWP respectively also indicate river deterioration. Stress tolerant taxa of Chironomidae, Oligochaeta, Simuliidae, and Hydropsychidae are also useful to evaluate river health.
- Comparison of metric results derived from post-monsoon and pre-monsoon sampling draw the conclusion that multimetric indices are applicable during the entire dry season.

Final conclusion and future prospects

The comparison of the findings in terms of substrate and current preferences with published ecological preferences from other bioregions were consistent in all cases. Only for very few taxa further preferred substrate types or current velocities have been observed in other regions. Likely, species of the investigated genera and families have a broader range of substrate and current preference. Since, all of the four metrics are inter-correlating, I suggest using only one metric to detect the effects of hydromorphological impact. The metric Lotic may also be able to indicate impacts of impoundments on the river ecosystem as it consists of taxa exhibiting preferences for faster current velocities. Impoundments lead to the reduction of flow velocity. Other ecological functions than substrate and current preferences are also used in other parts of the world to indicate anthropogenic influences, e.g. feeding strategies, ecological potential to alterations of water temperature and oxygen concentration. Currently, this ecological information is not sufficiently available for the Hindu Kush-Himalaya region. In addition, future investigations should focus at species level to obtain useful ecological information; niche concept works on species level, although this thesis showed that ecological allocations are also possible at the genus and family level.

A multimetric index was developed for all five stream types investigated to detect river deterioration caused by human impacts, mainly organic pollution and eutrophication. Results obtained for the Himalayan Subtropical Pine Forest are based on a large set of sampling sites and allow for full application of four robust core metrics. In the Eastern Himalayan Broadleaf Forest (34 sampling sites), the Western Himalayan Broadleaf Forest (17 sampling sites), and the Upper Gangetic Plains (18 sampling sites) the number of sampling sites were relatively low. Hence, relations by chance between environmental gradients and metrics could have occurred; however, all metrics selected are justified by ecological principles and also used worldwide for biomonitoring in other regions. To prove effectiveness of these metrics, additional sites should be investigated. I detected only one robust metric in the Lower Gangetic Plains; most likely because of the lack of reference sites. Although sampling for the multimetric index calculation seems possible during the entire dry season sampling should be carried out during the pre-monsoon season at times of minimum discharge regimes, maximum temperature and pollutant concentration.

Ecological assessment of river ecosystem health with benthic macroinvertebrates requires knowledge of life-cycle, ecological demands and ecological functions of the biota with respect to ecosystem processes. This thesis revealed insight in substrate and current preferences of benthic macroinvertebrates as impact indicators of hydromorphological degradation, and developed an ecological river assessment tool. The findings may be used for integrative river ecosystems assessment.

Finally, the thesis makes a small contribution of exploring riverine life of the Hindu Kush-Himalaya. The knowledge of river ecosystems still is scarce and riverine life and its interrelation within the community and with the environment, especially with various anthropogenic impacts, raises many more questions.

4 Kurzfassung

Einleitung

Die Fließgewässer der Hindu Kush-Himalaya Region werden von Menschen auf vielfältigste Weise genutzt und belastet. Hauptbelastungsfaktoren sind Einleitungen unbehandelter Industrie- und Haushaltswässer, Landnutzung im Einzugsgebiet und Stauungen (SOE Nepal 2001, SOE Bangladesch 2002, SOE Indien 2002, PCRWR 2002, SOE Bhutan 2004, ICIMOD/ASSESS-HKH 2005). In den Ländern Bangladesch, Bhutan, Indien, Nepal und Pakistan werden Fließgewässer überwiegend durch die Kontrolle von chemisch-physikalischen Größen, dem biologischen Sauerstoffbedarf (BOD), der Anzahl coliformer Bakterien und von human pathogenen Stoffen überwacht (Pradhan *et al.* 2005). Nur in Indien und in Nepal werden lokal Bewertungsmethoden angewandt, welche die Lebensgemeinschaft im Gewässer berücksichtigen (Central Pollution Control Board 1999, Sharma & Moog 2005, Nesemann *et al.* 2007). Diese Methoden basieren auf dem ASPT und BMWP System (Armitage *et al.* 1983), welche an die lokalen Verhältnisse angepasst wurden. Beide Methoden basieren auf der Einstufung von Taxa gegenüber organischer Belastung. In keinem der oben genannten Länder gibt es aktuell eine auf dem Makrozoobenthos basierende Bewertungsmethode, die eine integrative Bewertung des Ökosystems durchführt, also versucht, die Auswirkungen verschiedener Belastungsfaktoren auf das Ökosystem zu erfassen. Die Grundlage für die Entwicklung eines solchen Bewertungssystems ist das Wissen über die ökologischen Ansprüche der Tiere und über Prozesse innerhalb des Ökosystems. Für das Makrozoobenthos der Hindu Kush-Himalaya Region ist dieses Wissen aktuell nur spärlich vorhanden (Zusammenfassung in: Dudgeon 1999, Yule & Sen 2004).

Ziele der Arbeit

Die vorliegende Arbeit untersucht das Makrozoobenthos in den Fließgewässern der Hindu Kush-Himalaya Region (Bangladesch, Bhutan, Indien, Nepal und Pakistan) in Bezug auf dessen Eignung für eine integrative Bewertung von Fließgewässerökosystemen. Sie hat zwei Ziele.

Erstens sollen durch substratspezifische Aufsammlungen und die Anwendung von statistischen Auswertungsmethoden Erkenntnisse zur Ökologie des Makrozoobenthos in den Flüssen der Hindu Kush-Himalaya Region erlangt werden, die geeignet sind, anthropogene Einflüsse auf das Ökosystem anzuzeigen. Substrat- und Strömungspräferenzen werden dafür im Detail untersucht.

Zweitens soll ein multimetrischer Index zur integrativen Bewertung von Fließgewässerökosystemen der Hindu Kush-Himalaya Region mit dem Makrozoobenthos entwickelt werden.

Während der Post-Monsun Saison 2005 und der Prä-Monsun Saison 2006 wurden in fünf verschiedenen Ökoregionen (WWF Global 2000 ecoregions, Olson *et al.* 2001) der Hindu Kush-Himalaya Region zwei unterschiedliche standardisierte Aufsammlungsmethoden an 198 Fließgewässern durchgeführt. Die Parameter Ökoregion, Einzugsgebietsgröße, Höhenlage und Hydrologie (permanent fließend, von Quellen gespeist) dient zur Definition einer groben Fließgewässertypologie (Moog & Sharma 2005a). Insgesamt werden fünf Fließgewässertypen definiert, die von verschiedenen Gruppen des ASSESS-HKH[1] Konsortiums besammelt wurden. Diese lassen sich grob in Tieflandgewässer (45-250 m über Normal Null) und Mittelgebirgsgewässer (500-2500 m) einteilen. Alle Proben wurden mit einem Kescher mit 500 µm Maschenweite und einer Besammlungsfläche von 25 cm x 25 cm genommen. Die aus den beiden Besammlungen stammenden Tiere wurden auf das niedrigste mögliche Niveau bestimmt, meist Gattung und Familie. Zur Erlangung autökologischer Informationen über das Makrozoobenthos wurde eine substratspezifische Aufsammlung an unbelasteten Probestellen durchgeführt. Insgesamt wurden 271

[1] ASSESS-HKH = Development of an assessment system to evaluate the ecological status of rivers in the Hindu Kush – Himalaya region. Das Projekt wurde von der Europäischen Union (contract number: INCO-CT-2005_003659) gefördert und hatte zum Ziel, mehrere biologische Methoden basierend auf dem Makrozoobenthos zur Überwachung der Fließgewässer der Hindu Kush – Himalaya Region zu entwickeln.

Proben genommen. Zusätzlich zur Besammlung wurden am beprobten Substrat, die Fließgeschwindigkeit, die Tiefe und die Entfernung zum Ufer aufgenommen. Für die Entwicklung des multimetrischen Index wurden auf Grundlage einer Multi-Habitat Besammlungsmethode (Barbour *et al.* 1999 und AQEM Konsortium 2002) 373 Proben genommen. Die Probestellen verteilten sich gleichmäßig auf ein Fünf-Klassen Bewertungssystem, das überwiegend Eutrophierung und organische Verschmutzung erfasst (Moog & Sharma 2005a). An jeder Probestelle wurden Umweltparameter aufgenommen, die organische Verschmutzung, Eutrophierung, hydromorphologische Degradation sowie die Landnutzung im Einzugsgebiet und der Aue beschreiben.

Im Folgenden werden die wichtigsten Methoden, Ergebnisse und deren Interpretation vorgestellt.

Substrat- und Strömungspräferenzen des Makrozoobenthos in den Flüssen der Hindu Kush-Himalaya Region und deren Eignung zur Feststellung von hydromorphologischer Fließgewässerdegradation

Es wurde untersucht, ob und zu welchem Anteil die aufgenommenen Umweltparameter (Substrattyp, Fließgeschwindigkeit, Tiefe, Entfernung zum Ufer) die Variabilität im Datensatz erklären. Dafür wurden auf Basis einer kanonischen Korrespondenzanalyse (CCA) die jeweiligen Erklärungsanteile der Umweltparameter getrennt ermittelt (partielle-CCA) und zudem mittels des Monte Carlo Permutationstest überprüft, ob diese Erklärungsanteile signifikant sind.

Die Art des Substrattyps hat den größten Erklärungsanteil an der Verteilung der Taxa im Fließgewässer. Die anderen untersuchten Parameter erklären aber auch signifikant die Variabilität im Datensatz und sind somit von Bedeutung.

Diese Ergebnisse werden unterstützt von vielen Untersuchungen, die einerseits zeigen, dass der Substrattyp von außerordentlicher Wichtigkeit für die Zusammensetzung der Makrozoobenthosfauna ist, anderseits andere Umweltfaktoren (biotische und abiotische) ebenfalls die Verteilung der Taxa im Gewässer entscheidend mitbestimmen (z.B. Cummins & Lauf

1968, Minshall & Minshall 1977, Statzner *et al.* 1988, Rempel *et al.* 2000, Buss *et al.* 2004).

Für die statistische Auswertung zur Ermittlung von signifikanten Substrat- und Strömungspräferenzen wurden nur solche Taxa berücksichtigt, die in mindestens zehn Proben und zusätzlich mit mindestens 30 Individuen vorkamen. Um die Wirkung von Massenvorkommen zu reduzieren, wurden die Individuenzahlen logarithmiert. Um signifikante Substrat- und Strömungspräferenzen nachzuweisen, wurde für lineare Zusammenhänge (z.B. Präferenz zu „großen Steinen“) der Spearman Rang Korrelationstest r ($r > 0{,}05$, Signifikanzniveau $p < 0{,}05$) und für unimodale Zusammenhänge (z.B. Präferenz zu „kleinen Steinen“) der t-value Biplot angewendet.

50 Taxa zeigen eine signifikante Bindung an bestimmte Substrattypen und/oder bestimmte Strömungsverhältnisse. 34 Taxa zeigen signifikante Präferenzen für Steine. Diese Gruppe setzt sich überwiegend aus Eintagsfliegen (Ephemeroptera) und Köcherfliegen (Trichoptera) zusammen. 12 untersuchte Mollusken Taxa zeigen eine deutliche Präferenz für schlammige und sandige Substrattypen. 11 Taxa zeigen deutliche Präferenzen für gemäßigte bis schnellere Fließgeschwindigkeiten (moderate: 11-30 cm/s). Diese Gruppe wird überwiegend von Köcherfliegen gebildet.

Die Ergebnisse wurden mit der zur Verfügung stehenden Literatur aus verschiedenen biogeografischen Regionen verglichen (Ulmer 1955, 1957, Lepneva 1964, Stewart & Stark 1993, Merrit & Cummins 1996, Wiggins 1996, Stauder 1999, Dudgeon 1999, Yule & Sen 2004, Nesemann *et al.* 2007, Euro-limpacs Konsortium 2008). Der Vergleich zeigt Konsistenz in allen Fällen. Für fünf Taxa konnte zum ersten Mal Datenmaterial geliefert werden. Für einige wenige Taxa nennt die Literatur zusätzliche Präferenzen als die ermittelten. Dies hängt wahrscheinlich damit zusammen, dass die Arten der untersuchten Familien und Gattungen, weltweit betrachtet, eine breiteren Präferenzbereich zeigen. Dementsprechend sind die Ergebnisse nur auf die untersuchten Regionen anzuwenden. Generell kann aus den Ergebnissen geschlossen werden, dass die vorgestellte Methode (Besammlung und Auswertung) genutzt werden kann, um Informationen zur Autökologie des Makrozoobenthos zu erlangen.

- Taxa, die eine signifikante Bindung an steinige Substrate zeigen, wurden zum Metric Lithal zusammengefasst. Signifikante Präferenzen zu Steinen wurden weiter unterteilt. Zum Metric Lithobiont gehören solche Taxa, die signifikant an Steine gebunden sind, aber auch noch auf anderen Substraten vorkommen. Zum Metric Lithobiont gehören solche, die ausschließlich auf Steinen zu finden sind. Taxa mit Präferenzen zu schnelleren Fließgeschwindigkeiten (Klasse 3-6) wurden zum Metric Lotic zusammengefasst.

34 Taxa sind zum Metric Lithal zusammengefasst worden. Davon sind 21 dem Metric Lithophil und 13 dem Metric Lithobiont zugeteilt worden. Alle drei Metrics werden überwiegend von Eintagsfliegen und Köcherfliegen gebildet. 11 Taxa sind zum Metric Lotic zusammengefasst worden.

Auch in anderen Teilen der Erde werden Substrat- und Strömungspräferenzen verschiedener Taxa zu Metrics zusammengeführt. Diese Metrics werden dann für die Fließgewässerbewertung herangezogen (Schmedtje & Colling 1996, Meier *et al.* 2006).

- Um zu testen, ob die neuen Metrics in der Lage sind, hydromorphologische Degradationen von Fließgewässern anzuzeigen, wurden diese auf einen Datensatz angewendet, der einen hydromorphologischen Gradienten widerspiegelt. Box und Whisker Plots und der Mann-Whitney U-Test wurden verwendet, um diese Fähigkeiten genauer zu prüfen.

Alle Metrics können die Effekte hydromorphologischer Degradation nachweisen. Die Umwandlung von Individuenzahlen in Abundanzklassen liefert dabei die besten Ergebnisse. Die Metrics Lithal, Lithophil und Lotic sind am besten geeignet, hydromorphologisch beeinträchtigte Probestellen von nicht beeinträchtigten zu unterscheiden. Für diese drei Metrics wurden Grenzwerte für beeinträchtigte Stellen und nicht beeinträchtigte Stellen definiert. Alle Metrics korrelieren untereinander.

Andere Untersuchungen zeigen ebenfalls, dass die untersuchten Metrics die Effekte hydromorphologischer Degradation in Fließgewässern feststellen können (Schmedtje 1995, Lorenz *et al.* 2004, Meier *et al.* 2006). Die Morphologie der Fließgewässer in der Hindu Kush-Himalaya Region wird durch den Menschen vielfältig verändert. Die neuen Metrics sind in der Lage die Effekte dieser Beeinträchtigungen mit abnehmenden Werten zu erkennen. Da die Metrics untereinander korrelieren, sollte zur Bewertung nur einer der drei „Besten“ benutzt werden. Der Metric Lotic sollte dahingehend getestet werden, ob er in der Lage ist, Stauungen von Fließgewässern und deren Einflüsse auf die Lebensgemeinschaft anzuzeigen.

- Signifikante Substrat- und Strömungspräferenzen wurden durch die Entwicklung eines 20-Punkte-Systems genauer beschrieben.

Die meisten Taxa erreichen die höchste Punktzahl für den Substrattyp „kleine Steine“ (6-20 cm). Bei drei Taxa verteilen sich die Punkte fast ausschließlich auf die Substrate „große Steine“ (20-40 cm) und „Blöcke/anstehender Fels“ (> 40 cm). Fünf Ephemeroptera Taxa kommen fast nur auf „kleinen Steinen“ vor. Die meisten Taxa zeigen deutliche Präferenzen für „moderate“ bis „deutliche“ Fließgeschwindigkeiten.

Dass „kleine Steine“ als bevorzugter Lebensraum dient, hängt wahrscheinlich damit zusammen, dass dieser Substrattyp viele Eigenschaften aufweist, die er sich mit anderen Substraten teilt. Die Oberfläche „kleiner Steine“ ähnelt der von „großen Steinen“ und „Blöcken/anstehendem Fels“ und bietet wie diese Weidegängern von Moosen und Algen Lebensraum. Sand, Feinkies und Detritus wird in den strömungsberuhigten Bereichen zwischen den „kleinen Steinen“ abgelagert, welche als zusätzliche Lebensräume und Futterquellen dienen. Zudem ist das Interstitial als Rückzugsgebiet vor Katastrophen und ebenfalls als Lebensraum gut erreichbar (vgl. Beauger *et al.* 2006). Dementsprechend sollte eine nachhaltige Fließgewässerbewirtschaftung den Lebensraum „kleine Steine“ besonders berücksichtigen. Diejenigen Taxa, die nur auf wenigen Substrattypen auftraten, eignen sich gut als Bioindikatoren, da sie Veränderungen in ihrem Lebensraum nicht durch das Ausweichen in andere Substrate kompensieren können.

Entwicklung eines multimetrischen Bewertungssystems für die integrative Beurteilung von Fließgewässerökosystemen der Hindu Kush-Himalaya Region basierend auf dem Makrozoobenthos

- Eine Hauptkomponentenanalyse (PCA) wurde durchgeführt, um die wichtigsten Umweltgradienten im Datensatz zu erkennen. Die Untersuchung wurde für die Tieflandgewässer und die Mittelgebirgsgewässer getrennt durchgeführt. Zudem erfolgte die Untersuchung jeweils für die Stressoren organische Verschmutzung, Eutrophierung, hydromorphologische Degradation und Landnutzung getrennt.

  Die Ergebnisse zeigen, dass im Tiefland und im Mittelgebirge überwiegend die gleichen Umweltparameter die Variabilität am besten erklären. Wichtige Gradienten sind (1) für organische Belastung der biologische Sauerstoffbedarf und die Anzahl coliformer Bakterien, (2) für Eutrophierung die Konzentration von Ortho-Phosphat, (3) für hydromorphologische Degradation der Anteil an hölzerner Ufervegetation und der Anteil von Uferbefestigungen und (4) für die Landnutzung der Landuse Index (Hering *et al.* 2006b).

- Insgesamt wurden für jede Probestelle 28 Metrics berechnet, die auf Korrelationen mit den wichtigsten Umweltgradienten geprüft wurden. Metrics, die mit den Umweltgradienten korrelieren, wurden als Candidate Metrics für das weitere Verfahren berücksichtigt. Der Spearman Rangkorrelationstest wurde angewandt, um monoton steigende Verhältnisse aufzudecken, Box und Whisker Plots sollten zeigen, ob die Metrics gestresste von ungestressten Stellen unterscheiden können. Die Korrelationsuntersuchungen wurden für jeden der fünf Fließgewässertypen und für jeden der vier Stressoren separat durchgeführt. Es wurden verschiedene Grenzwerte definiert, damit Metrics als Candidate Metrics eingestuft wurden. Zusätzlich sollten die Korrelation zwischen ausgewählten Candidate Metrics und Umweltparametern ökologisch begründbar sein. Dann wurde geprüft, ob die so ausgewählten Candidate Metrics untereinander korrelieren. Nach festgelegten Regeln erfolgte die Auswahl der am besten geeigneten Metrics. Zum Schluss wurden diejenigen Candidate Metrics zu Core Metrics gewählt, die unter Referenzbedingungen robuste, replizierbare Ergebnisse liefern (75% der standardisierten Metric Werte in

Referenzstellen > 0,5). Die so für jeden Fließgewässertyp ermittelten Core Metrics wurden für die Entwicklung des multimetrischen Index berücksichtigt. Der Mittelwert aus allen Core Metrics ergibt den multimetrischen Index an einer Probestelle. Es wurden vier Klassengrenzen für die Beurteilung des jeweils errechneten Index definiert. Der Grenzwert für das Erreichen der Klasse „high“ ergibt sich aus den Mittelwerten aller 25% Perzentile der Core Metrics unter Referenzbedingungen. Die nachfolgenden Klassengrenzen werden gleichmäßig auf die nach Festlegung der Klassengrenze „high“ übrig gebliebene Spannweite verteilt.

Für den Fließgewässertyp *Himalayan Subtropical Pine Forest* wurden die Metrics Anteil EPT[2] Familien, Margalef Diversität, Evenness Diversität und ASPT[3] als robuste Core Metrics ermittelt. Diese zeigen in diesen Fließgewässertyp hauptsächlich organische Verschmutzung und Eutrophierung an. Sechs Core Metrics sind für den Fließgewässertyp *Western Himalayan Broadleaf Forest* ausgewählt worden. Die Metrics Anteil Plecoptera Familien, Anteil EPT Familien, Margalef Diversität, ASPT und Häufigkeit Baetidae-Simuliidae-Hydropsychidae-Chironomidae sind geeignet, die Folgen von Eutrophierung, hydromorphologischer Degradation und Landnutzung im Einzugsgebiet auf das Fließgewässer zu integrieren. Der Fließgewässertyp *Eastern Himalayan Broadleaf Forest* besitzt ebenfalls sechs robuste Core Metrics, nämlich Anzahl Ephemeroptera Familien, Shannon-Wiener Diversität, ASPT, Häufigkeit Oligochaeta, Häufigkeit Chironomidae und Häufigkeit Lithophile Taxa. Diese Metrics integrieren die Auswirkungen aller untersuchten Stressoren. Für den Fließgewässertyp *Upper Gangetic Plains* wurden Anteil Epheme-

[2] EPT = Ephemeroptera, Plecoptera, Trichoptera Taxa

[3] ASPT = Average Score Per Taxon (Armitage *et al.* 1983); Es wurde der ASPT angewendet, der an die Fließgewässer Nepals angepasst war (Sharma & Moog 2005).

roptera Familien, Shannon-Wiener Diversität und BMWP[4] als robuste Metrics ermittelt, welche hydromorphologische Degradation und Landnutzung im Einzugsgebiet anzeigen. Nur ein robuster Core Metric (Anteil Oligochaeta) konnte für den Fließgewässertyp *Lower Gangetic Plain* ermittelt werden. Dieser zeigt organische Belastung an. Für diesen Typ wurden zusätzlich die Candidate Metrics Anteil EPT Familien und BMWP für die Bildung des multimetrischen Index berücksichtigt.

Für alle fünf untersuchten Fließgewässertypen wurde ein multimetrischer Index entwickelt. Die zur Verfügung stehenden Core Metrics werden weltweit in multimetrischen Bewertungsmethoden zur Bewertung von Fließgewässern genutzt (z.B. Barbour *et al.* 1999 (Nordamerika), Maxted *et al.* 2000 (Nordamerika), Sandin & Hering 2004 (Europa), Ollis *et al.* 2006 (Süd-Afrika), Baptista *et al.* 2007 (Südamerika). Die Resultate stützen sich für den *Himalayan Subtropical Pine Forest* auf eine große Anzahl von Probestellen und sollten somit eine gesicherte Anwendung des multimetrischen Index garantieren. Für die Ökoregionen *Eastern Himalayan Broadleaf Forest* (34 Probestellen), *Himalayan Subtropical Pine Forest* (17 Pobestellen) und *Upper Gangetic Plains* (18 Probestellen) ist der Datensatz relativ klein. Folglich könnten Zufallskorrelationen zwischen den Umweltparameter und den Metrics aufgetreten sein. Wie auch immer, die Auswahl der Core Metrics ist konsistent mit ökologischen Prinzipien, und die Metrics werden weltweit zur biologischen Überwachung von Fließgewässern genutzt. In Zukunft sollte die Effektivität der Core Metrics für die drei letztgenannten Fließgewässertypen durch zusätzliche Untersuchungen überprüft werden. Für den Fließgewässertyp *Lower Gangetic Plain* konnte nur ein robuster Core Metric ermittelt werden. Dies liegt wahrscheinlich an einem Mangel an Referenzstellen im Tiefland.

[4] BMWP = Biological Monitoring Working Party (Armitage *et al.* 1983); Es wurde der BMWP angewendet, der an die Fließgewässer Nepals angepasst war (Sharma & Moog 2005).

- Es wurde getestet, ob sich die Metric Werte zwischen der Post-Monsun und Prä-Monsun Saison signifikant unterscheiden, oder ob eine Besammlung theoretisch über die gesamte Trockenperiode möglich ist. Als Signifikanztest wurde der Mann-Whitney U-Test verwendet. Für die Untersuchung wurden nur Metric Werte von Referenzstellen berücksichtigt, um eine Beeinflussung der Werte durch anthropogene Einflüsse auszuschließen.

  Von den 22 untersuchten Metrics zeigen nur zwei signifikante Unterschiede zwischen den Post-Monsun und den Prä-Monsun Werten.

  Brewin *et al.* (2000) untersuchte in Nepal die saisonalen Einflüsse des Monsuns auf die Häufigkeit des Makrozoobenthos. Seine Ergebnisse an Flüssen (500-2500 m) zeigen ebenfalls, dass sich die Häufigkeiten des Makrozoobenthos in ungestörten Fließgewässerabschnitten nicht ändern während der Trockenperiode. In der vorliegenden Arbeit gibt es zwei Ausnahmen, die Metrics Anteil an EPT Individuen und Häufigkeit Baetidae-Simuliidae-Hydropsychidae-Chironomidae schwanken signifikant im Fließgewässertyp Western Himalayan Broadleaf Forest. Bei genauer Betrachtung erkennt man aber für den Metric Anteil an EPT Individuen, dass sich die Interquartil Bereiche der Box und Whisker Plots für die Post- und Prä-Monsun Werte trotzdem stark überlappen; die Anteile also vergleichbar sind in den beiden Monsun Zeiten. Die erhöhten Werte des Metrics Häufigkeit Baetidae-Simuliidae-Hydropsychidae-Chironomidae in der Post-Monsun Phase kann durch schnelle Entwicklungsraten bedingt sein, die zu mehr als nur einer Generation innerhalb einer Trockenperiode führt.

Die vorliegende Arbeit leistet einen kleinen Beitrag auf den Weg zu einer nachhaltigen Bewirtschaftung der Fließgewässer in der Hindu Kush-Himalaya Region. Einerseits sind Substrat- und Strömungspräferenzen für einige Makrozoobenthos Taxa beschrieben, die für die Bewertung von Fließgewässern genutzt werden können, anderseits wurde ein multimetrisches Bewertungssystem entwickelt, welches eine integrative Bewertung der Fließgewässerökosysteme ermöglicht.

References

Ahmad, S. H. & A. K. Singh. 1989. A Comparative Study of the Benthic Fauna of Lentic and Lotic Water Bodies in Around Batna (Bihar), India. J. Environ. Biol. 10 (3): 283-291.

AQEM Consortium. 2002. Manual for the application of the AQEM method. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the water Framework Directive. Available from http://www.aqem.de/mains/products.php, Last update 10/10/2006.

Armitage, P. D., Moss, D. Wright, J. F. & M. T. Furse. 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water Res. 16: 1465-1477.

Bailey, R. C., Norris, R. H. & T. B. Reynoldson 2004. Bioassessment of freshwater ecosystems: Using the reference condition approach. New York, Springer.

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P. & J. L. Nessimian. 2007. A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. Hydrobiologia 575(1): 83-94.

Barbour, M. T., Norton, S. B. Thornton, K. W. & H. R. Preston (eds.). 2004. Ecological assessment of aquatic resources: Linking science to decision-making. SETAC Press, Pensacola, FL.

Barbour, M. T., Gerritsen, B. D., Synder, B. D. & J. B. Stribling. 1999. Rapid bioassessment protocols for use in wadable streams and rivers: periphyton, benthic macroinvertebrates and fish, 2nd edition. USEPA, Office of Water, Washington, D.C.

Barbour, M. T., Gerritsen, J., Griffith, G. E., Frydenborg, R., Mc Carron, E., White, J. S., & M. L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society 15: 185-211.

Barbour, M. T., Stribling, J. B. & J. R. Karr. 1995. Multimetric Approach for Establishing Biocriteria and Measuring Biological Condition. In: Davis, S. D. & T. P. Simon (eds.). Biological Assessment and Criteria. Tools for Water Resource Planning and Decision Making. Lewis Publisher, Boca Raton, London, Tokyo. 63-77.

Beauger, A., Lair, N., Reyes-Marchant, R. & J.-L. Peiry. 2006. The distribution of macroinvertebrate assemblages in a reach of the river Allier (France), in relation to riverbed characteristics. Hydrobiologia 571: 63-76.

Birk, S. &. D. Hering. 2002. Waterview Web-Database: a comprehensive review of European assessment methods for rivers. FBA News 20: 4.

Böhmer J., Rawer-Jost, C. & A. Zenker. 2004. Multimetric assessment of data provided by water managers from Germany: Assessment of several different types of stressors with macrozoobenthos communities. Hydrobiologia 516: 251-228.

Bournaud, M., Tachet, H. & A. L. Roux. 1987. The effects of seasonal and hydrological influences on the macroinvertebrates. Archiv für Hydrobiologie, Supplement 76: 25-51.

Brewin, P. A., Buckton, S. T. & S. J. Ormerod. 2000. The seasonal dynamics and persistence of stream macroinvertebrates in Nepal: Do monsoon floods represent disturbance? Freshwater Biology 44(4): 581-594.

Brewin, P. A., Newman, T. M. L. & S. J. Ormerod. 1996. Patterns of macroinvertebrate distribution in relation to altitude, habitat structure and land-use in streams of the Nepalese Himalaya. Archiv für Hydrobiologie 135: 79-100.

Brooks, A. J., Haeusler, T., Reinfelds, I. & S. Williams. 2005. Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles. Freshwater Biology 50: 331-341.

Buffagni, A., Armanini, D. G., Cazzola, M., Alba-Tercedor, J., López-Rodríguez, M. J., Murphy, J., Sandin, L. & Schmidt-Kloiber, A. 2007. Ephemeroptera Indicator Database. Eurolimpacs project (contract no. GOCE-CT-2003-505540), Workpackage 7 - Indicators of ecosystem health, Task 4. www.freshwaterecology.info. Version 3.2-08/2008, Last update: 24/01/2009.

Buss, D. F. & F. F. Salles. 2007. Using Baetidae species as biological indicators of environmental degradation in a Brazilian river basin. Environmental Monitoring and Assessment 130(1-3): 365-372.

Buss, D. F., Baptista, D. F., Nessimian, J. L. & M. Egler. 2004. Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in neotropical streams. Hydrobiologia 518: 179-188.

Buss, D. F., Baptista, D. F., Silveira, M. P., Nessimian J. L. & L. F. Dorvillé. 2002. Influence of water chemistry and environmental degradation on macroinvertebrate assemblages in a river basin in south-east Brazil. Hydrobiologia 481(1): 125-136.

Cairns, J. & R. Pratt. 1993. A history of biological monitoring using benthic invertebrates. In: Rosenberg, M. & V. R. Resh (eds). Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York. 10-27.

Casey, R. J. & H. F. Clifford. 1989. Colonization of natural substrata of different roughness and colour by Ephemeroptera nymphs using retrieval and direct observation techniques. Hydrobiologia 173: 185-192.

Central Pollution Control Board (editor). 2003a. Report on Survey of the River Yamuna and its Pollution Sources - a 245 km stretch between Hathnikund - Delhi. Ministry of Environment & Forest. New Delhi.

Central Pollution Control Board (editor). 2003b. Water Quality in India: Status and Trends (1999 - 2000). Ministry of Environment & Forest. New Delhi.

Central Pollution Control Board (editor). 1999. Bio-mapping of rivers. Parivesh. Ministry of Environment & Forest. New Delhi.

Chowdhary, S. K. 1984. Studies on bio-ecology of aquatic insects of Sind and Lidder streams of Kashmir. Indian J. of Ecology 11: 160-165.

Cummins, K. W. & G. H. Lauff. 1968. The influence of substrate particle size on the microdistribution of stream macrozoobenthos. Hydrobiologia 34: 145-181.

Davis, W. S. & T. P. Simon (eds.).1995. Biological assessment and criteria. Tools for water resource planning and decision making. Lewis Publishers, Boca Raton, London, Tokyo.

Dobriyal, A. K., Kumar, N., Kotnala, C. B., Bahuguna, A. K. & H. R. Singh. 1992. Preliminary observations on seasonal trends in macro-zoobenthic diversity in the river Nayar of Garhwal Himalaya. In: K. L. Sehgal (editor). Nat. Workshop on Res. and Dev. Need. Coldwater Fisheries. Today & Tomorrow's Printers & Publishers, New Delhi. 119-127.

Doledec, S., Statzner, B. & M. Bournard. 1999. Species traits for future biomonitoring across ecoregions: patterns along a human-impacted river. Freshwater Biology 42: 737-758.

Dudgeon, D. 2003. The contribution of scientific information to the conservation and management of freshwater biodiversity in tropical Asia. Hydrobiologia 500: 295-314.

Dudgeon, D. 1999. Tropical Asian streams. Zoobenthos, Ecology and Conservation. Hong Kong University Press, Hong Kong.

DWSS (Department of Water Supply and Sewerage). 2002. National Drinking Water Quality Standards. National Task Force on Drinking Water Quality Standards (editor). Government of Nepal. Kathmandu.

Eurolimpacs Consortium. 2008. Freshwaterecology.info - The Taxa and Autecology Database for Freshwater Organisms. Available from www.freshwaterecology.info. Version 3.2-08/2008, Last update: 24/01/2009.

Gjerløv, C., Hildrew, A. G. & J. I. Jones. 2003. Mobility of stream invertebrates in relation to disturbance and refugia: A test of habitat templet theory. Journal of the North American Benthological Society 22: 207-223.

GON / UNDP (Government of Nepal / United Nations Development Program). 2002. Progress report 2002 - Millennium Development Goals.

Graf, W., Lorenz, A., Tierno de Figueroa, J. M., Lücke, S., López-Rodríguez, M. J., Murphy, J. & A. Schmidt-Kloiber. 2007. Plecoptera Indicator Database. Eurolimpacs project (contract no. GOCE-CT-2003-505540), Workpackage 7 - Indicators of ecosystem health, Task 4. Available from www.freshwaterecology.info. Version 3.2-08/2008, Last update: 24/01/2009. (Appendix 1_5).

Graf, W., Murphy, J., Dahl, J., Zamora-Muñoz, C., López-Rodríguez M. J. & A. Schmidt-Kloiber. 2006. Trichoptera Indicator Database. Eurolimpacs project (contract no. GOCE-CT-2003-505540), Workpackage 7 - Indicators of ecosystem health, Task 4. Available from www.freshwaterecology.info. Version 3.2-08/2008, Last update: 24/01/2009.

Gupta, A. R. & G. Michael. 1992. Diversity, distribution, and seasonal abundance of Ephemeroptera in streams of Meghalaya State, India. Hydrobiologia 228: 131-139.

Gurtz, M. E., & J. B. Wallace. 1984. Substrate-mediated response of stream invertebrates to disturbance. Ecology 65: 1556-1569.

Hawkins, C. P. 1984. Substrate associations and longitudinal distributions in species of Ephemerellidae (Ephemeroptera: Insecta) from Western Oregon. Freshw. Invertebr. Biolo. 23: 181-188.

Hellawell, J. M. 1986. Biological indicators of freshwater pollution and environmental management. Elsevier, London.

Hering, D., Feld, C. K., Moog, O. & T. Ofenböck. 2006a. Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566(1): 311-324.

Hering, D., Johnson, R. K. Kramm, S. Schmutz, S. Szoszkiewicz, K. & P. F. M. Verdonschot. 2006b. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshwater Biology 51(9): 1757-1785.

Hering, D., Moog, O., Sandin, L. & P. F. M. Verdonschot. 2004a. Overview and application of the AQEM assessment system. Hydrobiologia 516: 1-20.

Hering, D., Meier, C., Rawer-Jost, C., Feld, C. K., Biss, R., Zenker, A., Sundermann, A., Lohse, S. & J. Böhmer. 2004b. Assessing streams in Germany with benthic invertebrates: selection of candidate metrics. Limnologica 34: 398-415.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20: 31-39.

Hodkinson, I. A. & J. K. Jackson. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environmental Management 35: 649-666.

Hoffmann, A. & M. Shrestha. 2008. The contribution of ASSESS-HKH to a sustainable water use in the Hindu Kush-Himalaya region. In: Moog, O., Hering, D., Sharma, S., Stubauer, I. & T. Korte (eds.). Conference proceedings. Rivers in the Hindu Kush-Himalaya - Ecology & Environmental Assessment. ASSESS-HKH consortium, Kathmandu, Nepal. 49-54.

Hutton, G. 2001. Economic evaluation and priority setting in water and sanitation interventions. In: Fewtrell, L. & L. Bartam (eds.). Water quality: Guidelines, standards and health. Assessment of risk and risk management for water-related infectious disease. IWA publications, London. 333-360.

ICIMOD / ASSESS-HKH (The International Centre for Integrated Mountain Development, Nepal / Development of an Assessment System to Evaluate the Ecological Status of Rivers in the Hind Kush-Himalaya region) (eds.). 2005. Review of river water policies, pressures and impacts, and socio-economic relevance. Available from http://www.assess-hkh.at/mains/products.php. Last update: 05/09/2008.

Jongman, R. H. G., Ter Braak, C. J. F. & O. F. R. Van Tongeren (eds). 2004. Data analysis in community and landscape ecology, 2nd edition. Cambridge University Press, Cambridge, New York, Melbourne, Madrid, Cape Town.

Kahlown, M. A. & M. Azam. 2004. Water quality issues and status in Pakistan. Pakistan Council of Research in Water Resources (PCRWR) (editor). Government document. Islamabad.

Karr, J. R. & E. W. Chu. 1999. Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington DC.

Kerans, B. L. & J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee valley. Ecological Applications 4(4): 768-785.

Kumar, K. 1987. Observations on seasonal variations of benthic organism in two trout streams of Kashmir. Proc. Indian nat. Sci. Acad. 53: 227-234.

Lancaster, J., Hildrew, A. G. & C. R. Townsend. 1990. Stream flow and predation effects on the spatial dynamics of benthic invertebrates. Hydrobiologia 203: 177-190.

Leopold, L. B. 1994. A View of the river. Harvard University Press, Cambridge.

Lepneva, S. G. 1964. Trichoptera. Zoological institute of the Academy of Sciences of the U.S.S.R. (editor). Vol. 88. Moskau, Leningrad.

Lorenz, A., Hering, D., Feld, C. K. & P. Rolauffs. 2004. A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. Hydrobiologia 516: 107-127.

Maxted, J. R., Barbour, M. T., Gerritsen, J., Poretti, J., Primrose, N., Silvia, A., Penrose, D. & R. Renfrow. 2000. Assessment framework for mid-Atlantic coastal plain streams using benthic invertebrates. Journal of the North American Benthological Society 19: 128-144.

Meier, C., Haase, P., Rolauffs, P., Schindehütte, K., Schöll, F., Sundermann, A. & D. Hering. 2006. Methodisches Handbuch Fließgewässerbewertung. Handbuch zur Untersuchung und Bewertung von Fließgewässern auf der Basis des Makrozoobenthos vor dem Hintergrund der EG-Wasserrahmenrichtlinie. http://www.fliessgewaesserbewertung.de. Access date: 16.02.2008.

Meren, A., Alfred, J. R. B. & A. Gupta. 1984. Studies on some lotic systems in the North-eastern Hill regions of India. Limnologica 15: 135-141.

Merrit, R. W. & K. W. Cummings (eds.). 1996. An introduction to the aquatic insects of North America, 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Merz, J., Nakarmi, G., Shrestha, S. K., Dahal, B. M., Dangol, P. M., Dhakal, M. P., Dongol, B. S., & R. Weingartner. 2003. Water: A scarce resource in rural watersheds of Nepal's Middle Mountains. Mountain Research and Development 23: 41-49.

Meschkowski, H. 1968. Wahrscheinlichkeitsrechnung. Bibliogr. Inst., Wien. Zürich.

Messerli, B. & J. D. Ives. 1997. Mountains of the World: A Global Priority. Parthenin. New York.

Minshall, G. W. 1984. Aquatic insect-substratum relationships. Pages 358-400. In: Resh, V. H. &. D. M. Rosenberg (eds.). The ecology of aquatic insects. Praeger, New York.

Minshall, G. W., & J. N. Minshall. 1977. Microdistribution of benthic invertebrates in a rocky mountain (U.S.A.) stream. Hydrobiologia 55: 231- 248.

Mohan, M., Bisht, R. S. & S. M. Das. 1989. Physico-chemical parameters in relation to aquatic entomofauna of river Bhagirathi and Bhilangana, Tehri-Garhwal, Himalaya. In: R. D. Khulbe (editor). Perspectives in Aquatic Biology. Papyrus Publishing House, Naini Tal, India. 197-203.

Moog, O. & S. Sharma. 2005a. Guidance for pre-classifying the ecological status of HKH rivers. - Working paper within ASSESS-HKH project. ASSESS-HKH consortium (editor). Available from http://www.assess-hkh.at/downloads/D10_Methodology.pdf. Last update: 05/09/2008.

Moog, O. & S. Sharma. 2005b. Description of simple operative top down stream typology for the HKH region- Working paper within ASSESS-HKH project. ASSESS-HKH consortium (editor). Available from http://www.assess-hkh.at/mains/products.php. Last update: 05/09/2008.

Moog, O. (editor). 2002. Fauna Aquatica Austriaca, Katalog zur autökologischen Einstufung aquatischer Organismen Österreichs. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft. Wien.

Morais, M., Pinot, P., Guilherme, P., Rosado, J. & I. Antunes. 2004. Assessment of temporary streams: the robustness of metric and multimetric indices under different hydrological conditions. Hydrobiologia 516: 229-249.

MOWR (Ministry of Water Resources) (editor). 2000. National Water Policy. Government of India. New Delhi.

Moya, N., Tomanova, S. & T. Oberdorff. 2007. Initial development of a multi-metric index based on aquatic macroinvertebrates to assess streams condition in the Upper Isiboro-Sécure Basin, Bolivian Amazon. Hydrobiologia 589(1): 107-116.

MWR/WEC (Ministry of Water Resources / Water and Energy Commission) (editor). 2005. National Water Plan, Water Resources Strategy Formulation. Phase III. in Kathmandu. Government document. Kathmandu.

National Task Force on Drinking Water Quality Standards (editor). 2005. A brief introduction on the final version of National drinking water qualitiy standards in Nepal. Report of the National Task Force on drinking water quality standards.

Negi, M. & H. R. Singh. 1990. Substratum as determining factor for bottom fauna in the river Alaknanda. Proc. Indian nat. Sci. Acad. 56: 417-423.

Nesemann H., Sharma, S., Sharma, G., Khanal, S. N., Pradhan, B., Shah, D. N. & R. D. Tachamo. 2007. Aquatic Invertebrates of the Ganga River System. Mollusca, Annelida, Crustacea (in part). H. Nesemann, Kathmandu.

Ofenböck, T., Moog, O., Gerritsen, J. & M. T. Barbour. 2004. A stressor specific multimetric approach for monitoring running waters in Austria using benthic macro-invertebrates. Hydrobiologia 516: 251-268.

Ollis, D. J., Dallas, H. F., Esler K. J. & C. Boucher. 2006. Bioassessment of the ecological integrity of river ecosystems using aquatic macroinvertebrates: An overview with a focus on South Africa. African Journal of Aquatic Science 31(2): 205-227.

Olson, D. M., Dinerstein, E., Wikramanaya, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E C., D'amico, J. A., Hollye, I. I., Morrison, S. J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H. Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P. & K. R. Kassem. 2001. Terrestrial ecoregions of the world: A new map of life on earth. BioScience 51(11): 933-938.

Ormerod, S. J., Rundle, S. D., Wilkinson, S. M., Daly, G. P., Dale, K. M. & I. Juttner. 1994. Altitudinal trends in diatoms, bryophytes, macroinvertebrates and fish of a Nepalese river system. Freshwater Biology 31: 309-322.

PCRWR (Pakistan Council of Research in Water Resources) (editor). 2002. Water quality status in Pakistan. Ministry of Science and Technology. Government document. Islamabad.

Pradhan, B. K., Siddhi, R. K., Shrestha, M. & R. Shilpakar. 2005. River quality policies, and pressures and impacts in the Hindu Kush-Himalaya region. Vol. I: Synthesis report. ASSESS-HKH consortium (editor). http://www.assess-hkh.at/mains/products.php. Last update: 05/09/2008. Access date: 27/01/2009.

Qadir, A. & R. N. Malik. 2009. Assessment of an index of biological integrity (IBI) to quantify the quality of two tributaries of river Chenab, Sialkot, Pakistan. Hydrobiologia. 621: 127-153.

Rawer-Jost, C., Böhmer, J., Blank, J. & H. Rahmann. 2000. Macroinvertebrate functional feeding group methods in ecological assessment. Hydrobiologia 422-423: 225-232.

Rempel, L. L., Richardson, J. S. & M. C. Healey. 2000. Macroinvertebrate community structure along gradients of hydraulic and sedimentary conditions in a large gravel-bed river. Freshwater Biology 45: 57-73.

Resh, V. H. 1995. Freshwater benthic macroinvertebrates and rapid assessment procedures for water quality monitoring in developing and newly industrialized countries. In: S. D. Davis & T. P. Simon (eds). Biological assessment and criteria. Tools for water resource planning and decision making. Lewis Publishers, Boca Raton, London, Tokyo. 167-180.

Resh, V. H., Brown, A. V., Covich, A. P., Gurtz, M. E., Li, H. G., Minshall, G. W., Reice, S. R., Sheldon, A. L., Wallace, J. B. & R. C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society 7: 433-455.

RGOB (Royal Government of Bhutan). 2004a. Environment Discharge Standard. Thimphu. National Environmental Commission (editor). Government document. Thimphu.

RGOB (Royal Government of Bhutan). 2004b. Environment Discharge Standard. National Environmental Commission (editor). Government document.

RGOB (Royal Government of Bhutan). 2002. Environmental Clearance and Environmental Assessment Strategic. National Environmental Commission (editor). Government document. Thimphu.

RGOB (Royal Government of Bhutan) (editor). 2000. Regulation for the Environmental Clearace of Projects and Regulation on Strategic Environmental Assessment. Government document. Thimphu.

Richardson, J. S. 1993. Limits to productivity in streams: Evidence from studies of macroinvertebrates. In: Gibson, R. J. & R. E. Cutting (eds.). Production of juveline Atlantic Salmon, Salmo salar, in natural waters. Canadian Special Publications of Fisheries and Aquatic Sciences Vol. 118.

Robinson, C. T. & G. W. Minshall. 1986. Effect of disturbance frequency on stream benthic community structure in relation to canopy cover and season. Journal of the North American Benthological Society 5: 237-248.

Rosenberg, M. & V. R. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, London.

Rundle, S., Jenkins, A. & S. J. Ormerod. 1993. Macroinvertebrate communities in streams in the Himalaya, Nepal. Freshwater Biology 30: 169-180.

Sandin, L. & D. Hering. 2004. Comparing macroinvertebrate indices to detect organic pollution across Europe: A contribution to the EC Water Framework Directive intercalibration. Hydrobiologia 516: 55-68.

SAWAN (South Asian Water Analysis Network). 2005. Proceedings of the Regional Integrated Workshop on Water Quality. In: Shrestha, M. & R. L. Shilpakar (eds.). Conference proceedings. Water Quality in South Asia: Issues and Status. International Center for Integrated Mountain Development (ICIMOD). Kathmandu.

Schmedtje, U. &. M. Colling. 1996. Ökologische Typisierung der aquatischen Makrofauna. Bayrisches Landesamt für Wasserwirtschaft (editor). Informationsberichte des Bayrischen Landesamtes für Wasserwirtschaft. Heft 4/96. München.

Schmedtje, U. 1995. Ökologische Grundlagen für die Beurteilung von Ausleitungsstrecken. Bayrisches Landesamt für Wasserwirtschaft (editor). 25. Bayrisches Landesamt für Wasserwirtschaft. München.

Shannon, C. E. & W. Weaver 1976. Mathemathische Grundlagen der Informationstheorie. Oldenbourg, München, Wien.

Sharma, S. & O. Moog. 2005. A reference based Nepalese Biotic Score and its application in the midland hills and lowland plains for river water quality as-sessment and management. In: Tripathi, R. D., Kulshrestha, K., Agrawal, M., Ahmed, K. J., Varsehen, C. K., Fripa, S. & P. Pushpangadan (eds.). Conference proceedings. Plant Response to Environmental Stress IBD, CO, Publisher, Lucknow.

Sharma, S., Bajracharya, R. M., Sitaula, B. K. & J. Merz. 2005. Water quality in the Central Himalaya. Current science 89: 774-786.

Sharma, R. C. 1986. The effect of physico-chemical factors on benthic fauna of Bhagirathi river, Garhwal Himalaya. Indian J. of Ecology 13: 133-137.

Silveira, M. P., Baptista, P. F., Nessimian, J. L. & M. Egler. 2005. Application of biological measures for stream integrity assessment in south-east Brazil. Environmental Monitoring and Assessment 101: 117-128.

Singh, M. & A. K. Singh. 2007. Bibliography of environmental studies in natural characteristics and anthropogenic influences on the Ganga river. Environmental Monitoring and Assessment 129: 421-432.

Singh, S. R. & V. K. Srivastava. 1989. Observations on the bottom fauna of the Ganga River (between Buxar and Ballia) with special reference to its role in the seasonal abundance of freshwater prawn (Macrobrachium birmanicum choprai (Tiwari). Acta Hydrochem. Hydrobiol. 17: 159-166.

SOE Bhutan (State of the Environment). 2004. Environmental issues. South Asia Cooperative Environment Programme (SACEP), Norwegian Agency for Development Cooperation (NORAD), United Nations Environment Programme (UNEP), National Environmental Commission (NEC), Royal Government of Bhutan (RGOB) (eds). Government document.

SOE Bangladesh (State of the Environment). 2002. Environmental issues. United Nations Environment Programme (UNEP) / Department of Energy / Government of Bangladesh (eds). Government document.

SOE India (State of the Environment). 2002. Environmental issues. United Nations Environment Programme (UNEP), South Asia Cooperative Environment Programme (SACEP) (eds). Government document.

SOE Nepal (State of the Environment). 2001. Environmental issues. South Asia Cooperative Environment Programme (SACEP), Norwegian Agency for Development Cooperation (NORAD), United Nations Environment Programme (UNEP) (eds.). Government document.

Sponseller, R. A., Benfield, E. F. & H. M. Valett. 2001. Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshwater Biology 46(10): 1409-1424.

Statzner, B., Hoppenhaus, K., Arens, M. F. & P. Richoux. 1997. Reproductive traits, habitat use and templet theory: A synthesis of world-wide data on aquatic insects. Freshwater Biology 38: 109-135.

Statzner, B., Gore, J. A. & V. Resh. 1988. Hydraulic stream ecology: Observed patterns and potential applications. Journal of the North American Benthological Society 7: 307-360.

Stauder, A. 1999. Bestand und Ökologie der Heptageniidae (Ephemeroptera) in den Fließgewässern des indischen Himalayas (Nord-West Indien). PhD thesis. Dissertation. Albert-Ludwigs-University. Freiburg.

Stewart, K. W. & B. P. Stark. 1993. Nymphs of North American Stonefly Genera (Plecoptera), 2nd edition. University of North Texas Press, Denton.

Suren, A. M. 1994. Macroinvertebrate communities of streams in western Nepal: effects of altitude and land use. Freshwater Biology 32: 323-336.

Ter Braak, C. J. F. & P. Smilauer. 2002. CANOCO. Reference manual and CanoDraw for windows user´s guide. Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA.

Thomson, J. R. 2002. The effects of hydrological disturbance on the densities of macroinvertebrate predators and their prey in a coastal stream. Freshwater Biology 47: 13-33.

Tomanova, S., Moya, N. & T. Oberdorff. 2008. Using macroinvertebrate biological traits for assessing biotic integrity of neotropical streams. River research and applications 24: 1230-1239.

Tomanova, S., Goitia, E. & J. Helesic. 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. Hydrobiologia 556: 251-264.

Townsend, C. R. & A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology 31: 265-275.

Ulmer, G. 1957. Köcherfliegen (Trichopteren) von den Sunda-Inseln, Teil 3, Larven und Puppen der Annulipalpia, unter Berücksichtigung verwandter Formen und deren Literatur aus anderen Faunengebieten. Archiv für Hydrobiologie, Suppl. 23: 109-470.

Ulmer, G. 1955. Köcherfliegen (Trichopteren) von den Sunda-Inseln, Teil 2, Larven und Puppen der Integripalpia, unter Berücksichtigung verwandter Formen und deren Literatur aus anderen Faunengebieten. Archiv für Hydrobiologie, Suppl. 21: 408-608.

Usseglio-Polatera, P., Bournaud, M., Richoux, P. & Tachet, H. 2000. Biomonitoring through biological traits of benthic macroinvertebrates: How to use species traits databases? Pages 153-162 in Hydrobiologia. Kluwer Academic Publishers.

Vlek, H. E., Verdonschot., P. F. M. & R. C. Nijboer. 2004. Towards a multimetric index for the assessment of Dutch streams using benthic macroinvertebrates. Hydrobiologia 516: 173-189.

Weigel, B. M., Henne, L. J. & L. M. Martinez-Rivera. 2002. Macroinvertebrate-based index of biotic integrity for protection of streams in west-central Mexico. Journal of the North American Benthological Society 21(4): 686-700.

WHO. 2008. World health statistics 2008 - Core Health Indicators. http://www.who.int/whosis/database/core/core_select_process.cfm. State 2008. Access date: 05/08/2008.

Wiggins, G. B. 1996. Larvae of North American caddisfly genera (Trichoptera), 2nd edition. University of Toronto Press, Toronto, Buffalo, London.

World Bank. 2006. Pakistan strategic country environmental assessment. Volume II: Technical annex, The cost of environmental degradation in Pakistan - An analysis of physical and monetary losses in environmental health and natural resources. South Asia Environment and Social Development Unit South Asia Region. Worl Bank Report No. 36946-PK.

Yule, C. M. & Y. H. Sen. 2004. Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lumpur.

Identification literature

All taxa

Dudgeon, D. 1999. Tropical Asian streams. Zoobenthos, ecology and conservation. Hong Kong University Press, Hong Kong.

Merrit, R. W. & K. W. Cummins. 1996. An introduction to the aquatic insects of North America, 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Morse, J. C., Yang, L. & L. Tian. 1984. Aquatic Insects of China useful for monitoring water quality. Hohai University Press, Nanjing.

Yule, C. M. & Y. H. Sen. 2004. Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lumpur.

Ephemeroptera

Allen, R. K. & G. F. Edmunds. 1963. New and little known Ephemerellidae from Southern Asia, Africa and Madagascar. Pacific Insects 5: 11-22.

Braasch, D. 1990. Neue Eintagsfliegenlarven aus Thailand, nebst einigen Bemerkungen zu deren generischen Status. Reichenbachia 28: 7-14.

Braasch, D. 1986. Zum Status der Gattung Heptagenia WALSH, 1863 in Indien. Reichenbachia 23: 131-134.

Braasch, D. 1984. Beitrag zur Kenntnis der Heptageniidae des Himalaya (III). Reichenbachia 22: 65-74.

Braasch, D. & T. Soldan. 1984. Zwei neue Arten der Gattung Cinygmina KIMMINS, 1937 aus Vietnam. Reichenbachia, Staatliches Museum für Tierkunde, Dresden 22: 195-202.

Soldan, T. & D. Braasch. 1984. Two new species of the genus Prosopistoma. Ephemeroptera. Acta ent. bohemoslov. 81: 370-376.

Stauder, A. 1999. Bestand und Ökologie der Heptageniidae (Ephemeroptera) in den Fließgewässern des indischen Himalayas (Nord-West Indien). PhD thesis. Dissertation. Albert-Ludwigs-University Freiburg.

Tomka, I. & A. Zurwerra. 1985. Key to the Genera of the Heptageniidae (Ephemeroptera) of the Holoarctic, Oriental and Ethiopian region. Entomologische Berichte Luzern 14: 113-123.

Wang, T. Q. & W. P. Mc Cafferty. 1995. First larval descriptions, new species, and evaluation, of the Southeast Asian genus Atopopus (Ephemeroptera, Heptageniidae). Bull. Soc. Hist. Nat., Toulouse 131: 19-25.

Zurwerra, A. I. T. 1985. Electrogena Gen. Nova, Eine neue Gattung der Heptageniidae. Entomologische Berichte Luzern 13: 99-104.

Plecoptera

Geijskes, D. C. 1952. Die Plekopteren der Deutschen Limnologischen Sunda-Expedition, nebst einigen Neubeschreibungen. Archiv für Hydrobiologie, Suppl. 21: 275-297.

Sivec, I. 1981. Contribution to the knowledge of Nepal stoneflies (Plecoptera). Aquatic Insects 3: 245-257.

Sivec, I., Stark, B. P. & S. Uchida. 1988. Synopsis of the World Genera of Perlinae (Plecoptera: Perlidae). SCOPOLIA 16: 1-66.

Stewart, K. W. & B. P. Stark. 1993. Nymphs of North American Stonefly Genera (Plecoptera), 2 edition. University of North Texas Press, Denton.

Trichoptera

Higler, L. W. G. 1992. A check-list of the Trichoptera recorded from India and a larval key to the families. Oriental Insects 26: 67-106.

Iwata, M. 1927. Trichopterous larvae from Japan. Annotationes zoologicae Japonenses 11: 203-233.

Lepneva, S. G. 1964. Trichoptera. Zoological institute of the Academy of Sciences of the U.S.S.R., Moskau, Leningrad.

Mathis, M. L. & D. E. Bowles. 1994. A description of the immature stages of Paduniella nearctica (Trichoptera: Psychomyiidae) with notes on its biology. Journal of New York Entomological Society 102(3): 361-366.

Prommi, T., Permkam, S. & H. Malicky. 2006. The immature stages of Pseudoleptonema quinquefasciatum MART. & P. supalak MAL. and CHANT. (Trichoptera: Hydropsychidae). Braueria 33: 26-30.

Ulmer, G. 1957. Köcherfliegen (Trichopteren) von den Sunda-Inseln, Teil 3, Larven und Puppen der Annulipalpia, unter Berücksichtigung verwandter Formen und deren Literatur aus anderen Faunengebieten. Archiv für Hydrobiologie, Suppl. 23: 109-470.

Ulmer, G. 1955. Köcherfliegen (Trichopteren) von den Sunda-Inseln, Teil 2, Larven und Puppen der Integripalpia, unter Berücksichtigung verwandter Formen und deren Literatur aus anderen Faunengebieten. Archiv für Hydrobiologie, Suppl. 21: 408-608.

Wiggins, G. B. 1996. Larvae of North American caddisfly genera (Trichoptera), 2nd edition. University of Toronto Press, Toronto, Buffalo, London.

Wiggins, G. B. 1969. Contributions to the biology of the Asian caddisfly family Limnocentropodidae (Trichoptera). Life Sci. Contr., R. Ont. Mus. 74: 1-29.

Appendix

Appendices are available on the enclosed CD-ROM

Substrate and Current preference

Appendix 1: A 1_1: Manual for substrate specific sampling

A 1_2: Identification key Ephemerellidae (Ephemeroptera)

A 1_3: Figures substrate preferences; individual numbers, abundance class values, 20 point allocation

A 1_4: Figures current preferences; individual numbers, abundance class values, 20 point allocation

A 1_5: Table comparison findings with literature

A 1_6: Description sampling code

A 1_7: Taxalists substrate specific sampling

A 1_8: Site protocols substrate specific sampling

Development of a Multimetric Index

Appendix 2: A 2_1: Description sampling sites India

A 2_2: Figures candidate metrics

A 2_3: PCA input sheets, lowlands

A 2_4: PCA input sheets, mountains

A 2_5: Taxalists Multi-Habitat sampling

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Erklärung

Hiermit erkläre ich, gem. § 6 Abs. 2, Nr. 6 der Promotionsordnung der Math.-Nat.-Fachbereiche zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbstständig verfasst und mich keiner als der angegebenen Hilfsmittel bedient habe.

Essen, den__________________ ____________________________

Erklärung

Hiermit erkläre ich, gem. § 6 Abs. 2, Nr. 7 der Promotionsordnung der Math.-Nat.-Fachbereiche zur Erlangung des Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema „River Assessment using Benthic Macroinvertebrates in the Hindu Kush-Himalaya Region - Substrate and Current Preferences, and Development of an Assessment Method“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Thomas Korte befürworte.

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